



## Identification of early life-history stages of Caribbean *Apogon* (Perciformes: Apogonidae) through DNA Barcoding

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### Abstract

Early life-history stages of 12 of 17 species of western Central Atlantic *Apogon* were identified using molecular data. A neighbor-joining tree was constructed from mitochondrial cytochrome oxidase-*c* subunit I (COI) sequences, and genetic lineages of *Apogon* in the tree were identified to species based on adults in the lineages. Relevant portions of the tree subsequently were used to identify larvae of *Apogon* species from Carrie Bow Cay, Belize, and juveniles from Belize and other western Central Atlantic localities. Diagnostic morphological characters of larvae and juveniles were investigated by examining preserved vouchers from which the DNA was extracted and digital color photographs of those specimens taken before preservation. Orange and yellow chromatophore patterns are the easiest and sometimes only means of separating *Apogon* larvae. Patterns of melanophores and morphometric features are of limited diagnostic value. For juveniles, chromatophore patterns and the developing dark blotches characteristic of adults are the most useful diagnostic features. Larvae were identified for *Apogon aurolineatus*, *A. binotatus*, *A. maculatus*, *A. mosavi*, *A. phenax*, *A. planifrons*, and *A. townsendi*. Juveniles were identified for those species (except *A. planifrons*) and for *A. pseudomaculatus*, *A. lachneri*, *A. pillionatus*, *A. robbyi*, and *A. quadrisquamatus*. One larval specimen occurs in an unidentified genetic lineage, and five adults occur in another unidentified genetic lineage. *Apogon* species can be divided into at least four groups based on pigmentation patterns in early life stages. Further investigation is needed to determine if those groups are meaningful in the generic classification of *Apogon* species.

**Key words:** COI, cardinalfishes, fish larvae, pigmentation patterns, chromatophores, Belize

### Introduction

The family Apogonidae is represented in the western Central Atlantic by three genera, *Apogon*, *Astrapogon*, and *Phaeoptyx*, and 23 species (Böhlke & Chaplin, 1993; Gon 2002). Early life stages of all species of *Phaeoptyx* and *Astrapogon* have been described (Lara 2006, Baldwin *et al.* 2009a), but less is known about young stages of *Apogon*. Lara (2006) provided brief descriptions and illustrations of early stages of ten species of western Atlantic *Apogon*, but most of the specimens that she was able to identify were juveniles. Although the compilation in which Lara's chapter was included—Richards' (2006) *Early Stages of Atlantic Fishes*—represents the best effort to date to concentrate information regarding early stages of western Central Atlantic fishes, it contains almost no information useful in identifying larval *Apogon*. DNA barcoding (Hebert *et al.* 2003) is emerging as a valuable tool for identifying larvae and juveniles of marine fishes (e.g., Pegg *et al.* 2006, Victor 2007, Baldwin *et al.* 2009a, Packer *et al.* 2009, Victor *et al.* 2009, Valdez-Moreno *et al.* 2010). Additionally, barcoding is useful for resolving complex taxonomic issues (e.g., Baldwin *et al.* 2009b) and for identifying cryptic new species of marine fishes (e.g. Victor 2007, Tornabene *et al.* 2010, Baldwin *et al.* 2011), both of which can aid in the identification of fish larvae and juveniles by providing a more complete picture of species diversity. Valdez-Moreno *et al.* (2010) matched larvae and adults of numerous marine fish species of Mexico through DNA barcoding, including those of *Apogon maculatus*. No descriptions of larvae were provided.

Ongoing studies at the Smithsonian Institution are utilizing DNA barcoding data in a reanalysis of species diversity of Caribbean shorefishes (e.g., Baldwin *et al.* 2009b, Tornabene *et al.* 2010, Baldwin *et al.* 2011) and in identifying net-collected fish larvae from the Institution's research station at Carrie Bow Cay, Belize (Baldwin *et al.* 2009a, Tornabene *et al.* 2010). The purposes of this paper are to identify genetic lineages of *Apogon* derived from DNA barcoding data of western Central Atlantic fishes; match early life stages of Belize *Apogon* to adults using the barcoding data; describe diagnostic morphological features of larval and juvenile *Apogon* identified in this study; provide comparative sections to help distinguish early-life stages of *Apogon* spp. from one another; and provide color photographs of larvae and juveniles to highlight distinctive color patterns that are lost upon conventional preservation. Additionally we comment on the potential utility of early life stages of western Atlantic *Apogon* in phylogenetic studies of the genus.

## Materials and Methods

Juveniles and adults were collected in Belize, Curaçao, Florida, and the Bahamas using the fish anesthetic quinaldine sulfate or the fish toxicant rotenone and dip nets. Larvae were collected in a plankton net of 505µm mesh fitted onto a 0.5 x 1 m rectangular frame made of PVC pipe and deployed from a dock at Carrie Bow Cay, Belize (16°48.5'N, 88°05'W). Juveniles were also obtained by rearing net-collected larvae in a flow-through seawater system. Selected specimens were measured to the nearest 0.5 mm, photographed with a Fuji FinePix 3 digital camera to record color patterns, sampled for genetic analysis, and, when the entire specimen was not used as a tissue sample, preserved as vouchers. Tissue sampling for molecular work involved removing a muscle biopsy, eye, or caudal body portion (depending on size and life stage) and storage in saturated salt buffer (Seutin *et al.* 1990). Genomic DNA was extracted from up to approximately 20 mg of minced preserved tissue via an automated phenol-chloroform extraction on the Autogenprep965 (Autogen, Holliston, MA) using the mouse-tail tissue protocol with a final elution volume of 50 µl. For PCR, 1 µl of this genomic DNA was used in a 10 µl reaction with 0.5U Bioline (Bio-Line USA, Boston, MA) taq polymerase, 0.4µl 50mM MgCl<sub>2</sub>, 1µl 10X buffer, 0.5µl of 10mM dNTPs, and 0.3µl of 10µM of each primer FISH-BCL (5'-TCAACYAATCAYAAAGATATYGGCAC) and FISH-BCH (5'-TAAACTTCAGGGTGACCAAAAAATCA). The thermal cycler program for PCR was one cycle of 5 m @ 95°C; 35cycles of 30 s @ 95°C, 30 s @ 52°C and 45 s @ 72°C; one cycle of 5 m @ 72°C, and a hold at 10°C. PCR products were purified with Exosap-IT (USB, Cleveland, OH) using 2 µl of 0.2x enzyme and incubated for 30 m @ 37°C. The reaction was then inactivated for 20 m @ 80°C. Sequencing reactions were performed using 1µl of this purified PCR product in a 10 µl reaction containing 0.5 µl primer, 1.75 µl BigDye buffer and 0.5 µl BigDye (ABI, Foster City, CA) and run in the thermal cycler for 30 cycles of 30 s @ 95°C, 30 s @ 50°C, 4 m @ 60°C and then held at 10°C. These sequencing reactions were purified using Millipore Sephadex plates (MAHVN-4550; Millipore, Billerica, MA) per manufacturer's instructions and stored dry until analyzed. Sequencing reactions were analyzed on an ABI 3730XL automated DNA sequencer, and sequence trace files were exported into Sequencher 4.7 (GeneCodes, Ann Arbor, MI). Using the Sequencher program, ends were trimmed from the raw sequences until the first and last 10 bases contained fewer than five base calls with a confidence score (phred score) lower than 30. After trimming, forward and reverse sequences for each specimen were assembled, each assembled contig was examined and edited by hand, and each sequence was checked for stop codons. Finally the consensus sequence from each contig was aligned and exported in a Nexus format. A neighbor-joining tree (Saitou & Nei 1987) and distance matrices were generated using MEGA 4 (Tamura *et al.* 2007) on an analysis of Kimura 2-parameter distances (Kimura 1980).

Photographs and voucher specimens of each species were examined to identify distinguishing morphological features for all life-history stages. Larvae and juveniles were examined for patterns of pigment and other diagnostic features. Based on preliminary surveys to assess characters that exhibit variation within the genus, the main characters taken into account were caudal-peduncle length (oblique distance between posterior base of anal fin and center of caudal-fin base) for larvae, and numbers of gill rakers on the lower limb of the first arch (including rudiments) for juveniles. When intact specimens were available as vouchers, measurements were made under a dissecting microscope fitted with an ocular micrometer. When no or only a partial voucher remained after tissue sampling for DNA analysis, measurements were made from photographs of the intact specimens taken prior to dissection. Measurements were made to the nearest 0.1 mm and % SL was rounded to the nearest mm. All photographs in figures

are of recently deceased specimens—i.e., before preservation. Transparent fins and fin membranes are often difficult to discern in original photographs of some larvae, and in several cases images of larvae were cut from their original background and placed on a new background using Adobe Photoshop. In those cases, as well as some in which background areas around the fins were cleaned up, shape of the fins and position of the membranes are approximations.

Until incipient melanophore patterns of adults (in those species that have them) appear ontogenetically—usually in juveniles, there are often no clear morphological differences between larval and juvenile stages. For purposes of consistency, we considered all specimens taken in the plankton net as pre-settlement-stage larvae and all other early life stages (collected with dip nets and chemicals or reared from net-collected larvae) as juveniles.

*Apogon* material examined is listed in Appendix 1. Many of the cataloged voucher specimens are only partial specimens because of the tissue sample taken for genetic analysis. For most specimens analyzed genetically, a digital color photograph is housed at the Smithsonian Institution. Cytochrome Oxidase 1 sequences for specimens analyzed genetically are deposited in GenBank with accession numbers JN827894-JN828087. The sequences also are part of the BOLD (Barcode of Life Data Systems) database (<http://www.boldsystems.org>), project name = “Apogon Paper.” Abbreviations used in DNA numbers reflect geographical location: BAH – Bahamas; BLZ – Belize; CUR – Curaçao; FCC (Florida Fish and Wildlife Conservation Commission), FWRI (Florida Fish and Wildlife Research Institute), and SMS (Smithsonian Marine Station at Ft. Pierce) – Florida; SAB – Saba Bank (Netherlands Antilles). Acronyms for catalog numbers are USNM (United States National Museum) and FSBC (Florida State Board of Conservation).

## Results

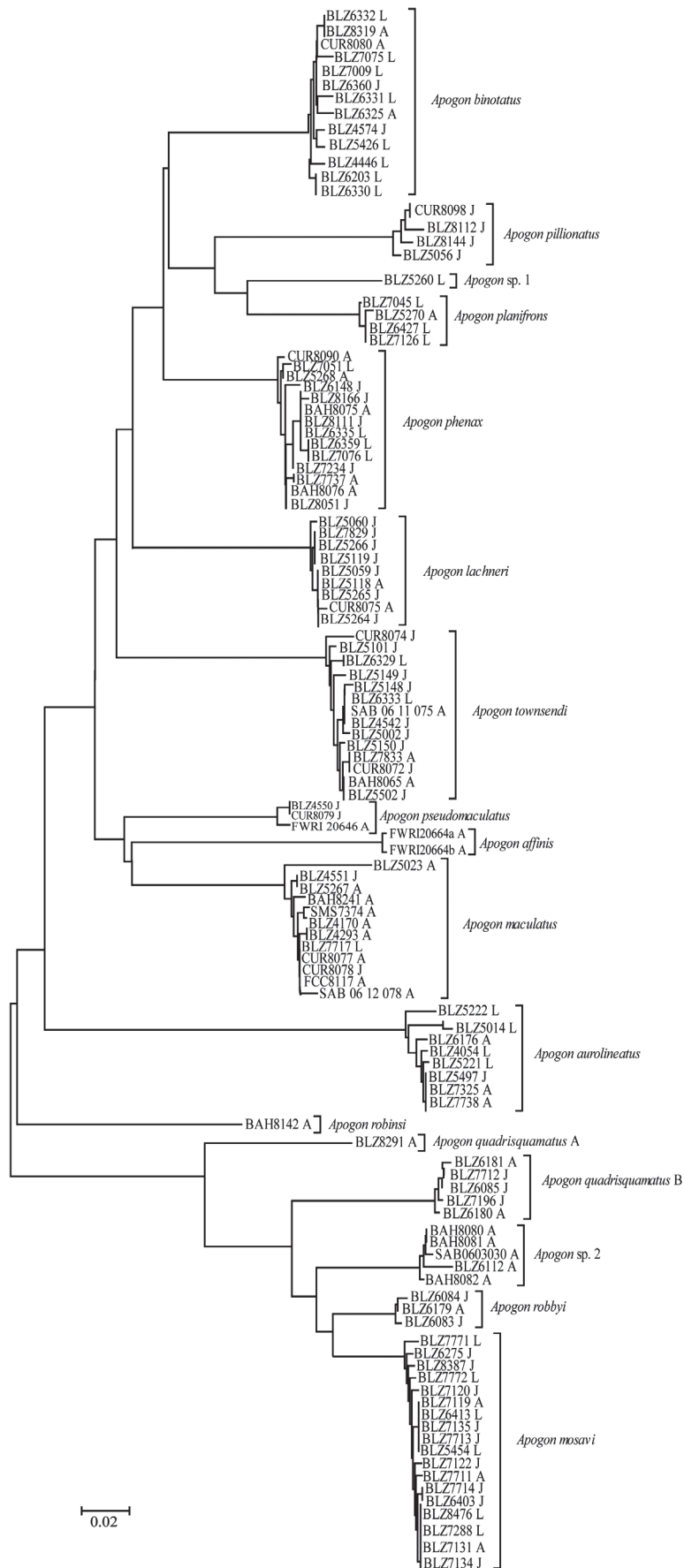
Seventeen genetic lineages of *Apogon* are represented in our material (Fig. 1). Thirteen of the 17 lineages were identified to species based on identification of adults in those lineages using previously published identification keys and descriptions (Böhlke & Chaplin 1993, Gon 2002). One lineage comprising only juveniles was identified to species (*A. pillionatus*) based on a process of elimination and comparative morphological examination (see “*A. pillionatus*” below). Two lineages were identified to the same species, *A. quadrisquamatus* (*A. quadrisquamatus* A and B in Fig. 1). Two lineages could not be identified: *Apogon* sp. 1 represented by a single larval specimen, and *Apogon* sp. 2 represented by five adult specimens. Larvae of seven and juveniles of 11 *Apogon* species were identified based on their position in the neighbor-joining tree (i.e., occurring in lineages identified based on adults). No early life-history stages of *Apogon affinis* and *A. robinsoni* are present in our material (Fig. 1), and because no adults of *A. evermanni*, *A. gouldi*, and *A. leptocaulus* were collected, those species do not appear in the tree. The unidentified genetic lineage comprising a single larval specimen could represent one of those or an undescribed species. DNA barcoding sequences clearly distinguish *Apogon* species from individuals based on high interspecific and low intraspecific genetic divergences (5–25% interspecific vs. 0–1% intraspecific, Table 1).

COI sequences from 196 *Apogon* individuals were generated in this study. Eighty of those sequences were removed from the Nexus file used to create Figure 1 to reduce the size of the tree so that it could be viewed as a single-page figure. Removal of those sequences had no effect on the overall topology of the tree. A table listing all material included in the analysis appears in Appendix 1. Inter- and intraspecific genetic divergences (Table 1) were calculated based on the complete data set.

Table 2 provides information on numbers of gill rakers on the lower limb of the first arch of western Central Atlantic *Apogon* species. Gill rakers are useful in separating juveniles of some *Apogon* species.

### *Apogon binotatus* (Poey)

**Identification.** Eleven adult specimens of *A. binotatus* provided the basis for genetic identification of larvae and juveniles (Appendix 1, one adult is shown in Fig. 2). Adult *A. binotatus* can be distinguished from other *Apogon* by the combination of eight segmented anal-fin rays, body and lateral-line scales of similar size, and body with two distinct dark markings posteriorly—a bar below the second dorsal fin and another on the posterior part of the caudal peduncle, both bars narrow, much deeper than wide (Böhlke & Chaplin 1993, Gon 2002).

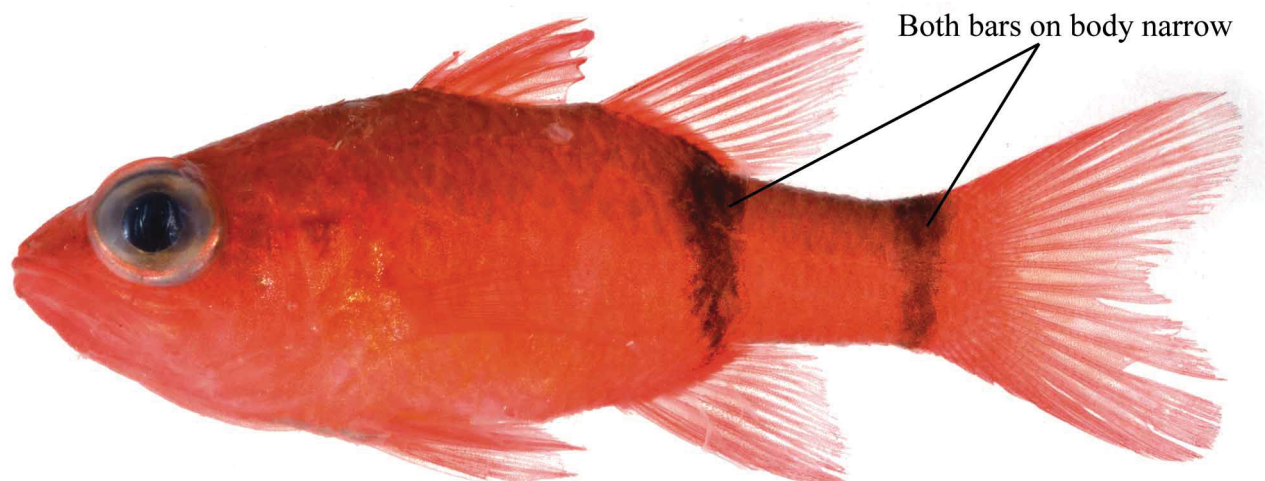


**FIGURE 1.** Neighbor-joining tree derived from mitochondrial cytochrome oxidase 1 sequences showing genetic lineages of *Apogon* species from Bahamas (BAH), Belize (BLZ), Curaçao (CUR), Florida (FCC, FWRI, SMS), and Saba Bank (SAB). L = larva, J = juvenile, A = adult.

**Table 1.** Average Kimura 2-parameter distance summary for *Apogon* species. Intraspecific averages in bold font. na = not applicable (one specimen).

	<i>A. aurolineatus</i>	<i>A. maculatus</i>	<i>A. phenax</i>	<i>A. binotatus</i>	<i>A. townsendi</i>	<i>A. pseudomaculatus</i>	<i>A. pillionatus</i>	<i>A. lachneri</i>	<i>A. planifrons</i>	<i>A. mosavi</i>	<i>A. robbyi</i>	<i>A. quadrisquamatus</i> Lineage A	<i>Apogon</i> sp.	<i>A. affinis</i>	<i>A. robbinsi</i>	<i>Apogon</i> sp. 2	<i>A. quadrisquamatus</i> Lineage B
<i>A. aurolineatus</i> (N = 12)	<b>0.01</b>																
<i>A. maculatus</i> (N = 18)	0.20	<b>0.01</b>															
<i>A. phenax</i> (N = 32)	0.19	0.13	<b>0.00</b>														
<i>A. binotatus</i> (N = 28)	0.21	0.14	0.10	<b>0.00</b>													
<i>A. townsendi</i> (N = 27)	0.24	0.15	0.10	0.13	<b>0.00</b>												
<i>A. pseudomaculatus</i> (N = 3)	0.22	0.12	0.10	0.14	0.14	<b>0.00</b>											
<i>A. pillionatus</i> (N = 5)	0.23	0.14	0.10	0.12	0.15	0.16	<b>0.01</b>										
<i>A. lachneri</i> (N = 11)	0.23	0.16	0.10	0.12	0.13	0.14	0.14	<b>0.00</b>									
<i>A. planifrons</i> (N = 6)	0.22	0.18	0.10	0.10	0.17	0.17	0.11	0.15	<b>0.00</b>								
<i>A. mosavi</i> (N = 36)	0.23	0.20	0.20	0.22	0.21	0.21	0.24	0.20	0.244	<b>0.00</b>							
<i>A. robbyi</i> (N = 3)	0.23	0.20	0.20	0.23	0.20	0.19	0.22	0.21	0.22	0.05	<b>0.00</b>						
<i>A. quadrisquamatus</i> Lineage A (N = 1)	0.25	0.22	0.20	0.24	0.22	0.18	0.23	0.20	0.24	0.09	0.10	<b>0.00</b>					
<i>Apogon</i> sp. (N = 1)	0.23	0.14	0.10	0.12	0.14	0.11	0.11	0.14	0.08	0.23	0.20	0.23	<b>na</b>				
<i>A. affinis</i> (N = 2)	0.24	0.14	0.10	0.17	0.15	0.14	0.16	0.16	0.18	0.18	0.20	0.21	0.16	<b>0.00</b>			
<i>A. robbinsi</i> (N = 1)	0.21	0.17	0.20	0.17	0.18	0.18	0.17	0.14	0.18	0.19	0.20	0.20	0.17	0.20	<b>na</b>		
<i>Apogon</i> sp. 2 (N = 5)	0.24	0.21	0.20	0.24	0.21	0.18	0.23	0.22	0.22	0.07	0.10	0.09	0.23	0.20	0.21	<b>0.00</b>	
<i>A. quadrisquamatus</i> Lineage B (N = 5)	0.23	0.17	0.20	0.20	0.19	0.17	0.20	0.22	0.21	0.12	0.10	0.14	0.20	0.20	0.18	0.09	<b>0.00</b>





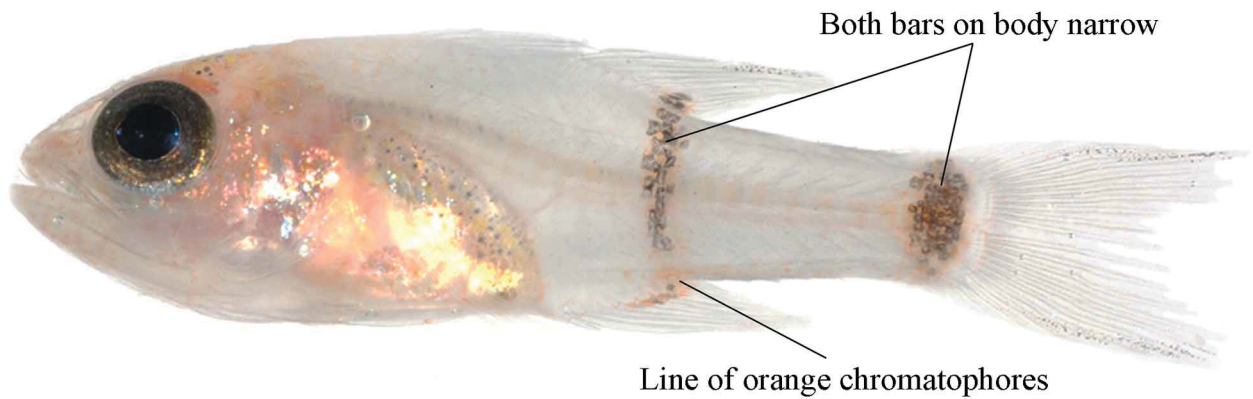
**FIGURE 2.** *Apogon binotatus*, adult, 46.0 mm SL, DNA # BLZ 6325, photograph by J. Mounts and C. Baldwin.

**TABLE 2.** Typical values and upper and lower extremes of numbers of gill rakers on the lower limb of the first arch in western Atlantic *Apogon* species. Data are from Böhlke and Chaplin 1968, Böhlke and Randall 1968, Dale 1977, Smith-Vaniz 1977, Randall and Böhlke 1981, Gilbert and Tyler 1997, and Gon, 2002.

Species	Typical Number of Gill Rakers on Lower Limb of First Arch	Upper and Lower Extremes of Gill Rakers on Lower Limb of First Arch
<i>A. affinis</i>	14	13–15
<i>A. aurolineatus</i>	10–11	9–12
<i>A. binotatus</i>	13	12–14
<i>A. evermanni</i>	16	15–17
<i>A. gouldi</i>	11–12	11–12
<i>A. lachneri</i>	16–17	16–17
<i>A. leptocaulus</i>	15–16	15–16
<i>A. maculatus</i>	14	13–16
<i>A. mosavi</i>	14–15	13–16
<i>A. phenax</i>	13–14	13–14
<i>A. pillionatus</i>	12	11–13
<i>A. planifrons</i>	15	14–16
<i>A. pseudomaculatus</i>	12	13–14
<i>A. quadrisquamatus</i>	13	12–14
<i>A. robbyi</i>	12–13	12–14
<i>A. robinsi</i>	17	16–18
<i>A. townsendi</i>	17	16–18

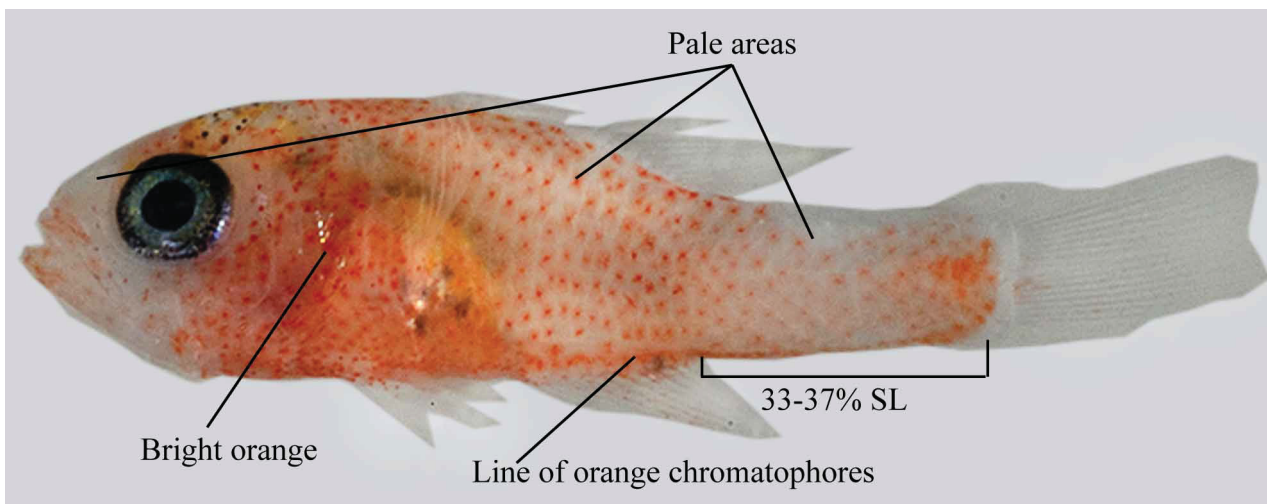
**Juveniles (Fig. 3).** Juveniles identified genetically range from 13.5 to 18.0 mm SL. The body is pale. The upper part of the head and the gut are pink. The fins are mostly clear, but there are melanophores on the tips of the anterior rays of the second dorsal and anal fins and on the outer rays of the caudal fin. There are numerous melanophores on top of the head and over the gut. There is a slender bar beneath the end of second dorsal-fin base and a wider bar on the caudal peduncle. There are 12–13 gill rakers on the lower limb of the first gill arch, counts consistent with values for adults (Table 2).

**Comparisons Among Juveniles.** Although adult *A. binotatus* are easily distinguished from other *Apogon* species by having both body bars slender (much deeper than wide), the posterior bar in juvenile *A. binotatus* is broader than the anterior bar. It is still narrow relative to the broad posterior bar in *A. townsendi*, *A. phenax*, and *A. pillionatus*. Juvenile *A. binotatus* is most similar to juvenile *A. phenax*, but it can be separated from that species by having the anterior bar slender (vs. at least slightly wedge-shaped in *A. phenax*) and positioned entirely beneath the second dorsal fin (vs. half or more of the bar behind the fin in *A. phenax*). The configuration of the two bars in juveniles of *A. binotatus* is sufficient to separate them from known juveniles of other *Apogon*.



**FIGURE 3.** *Apogon binotatus*, juvenile, 14.0 mm SL, DNA # BLZ 4574, photograph by J. Mounts and C. Baldwin.

**Larvae (Fig. 4).** *Apogon binotatus* larvae analyzed genetically range from 8.5 to 11.0 mm SL. The body is orange to pale orange, and there may be clear (or paler orange) areas on the snout just anterior to the eye, on the caudal peduncle, and near the middle of the trunk. In one 9.0-mm SL specimen there is a wide pale area posterior to the second dorsal fin and anterior to the caudal-fin base. The fins are clear except for a few orange spots along the posterior base of the anal fin and a few spots in the center of the caudal-fin base. There is a line of bright orange pigment on ventral side of the body from the anal fin to the caudal peduncle. There are melanophores on top of the head, in the temporal region, and over the swimbladder. Some specimens have a few melanophores on the lateral surface of the gut. The caudal-peduncle length ranges from 33–37% SL.



**FIGURE 4.** *Apogon binotatus*, larva, 9.0 mm SL, DNA # BLZ 6331, photograph by J. Mounts and C. Baldwin.

**Comparisons Among Larvae.** Fresh specimens of *A. binotatus* larvae are extremely similar to *A. phenax* larvae, but they can often be separated by snout pigment (no or pale orange spot anteriorly in *A. binotatus* vs. usually a prominent orange spot on snout in *A. phenax*). From *A. planifrons*, larval *A. binotatus* differs in lacking yellow

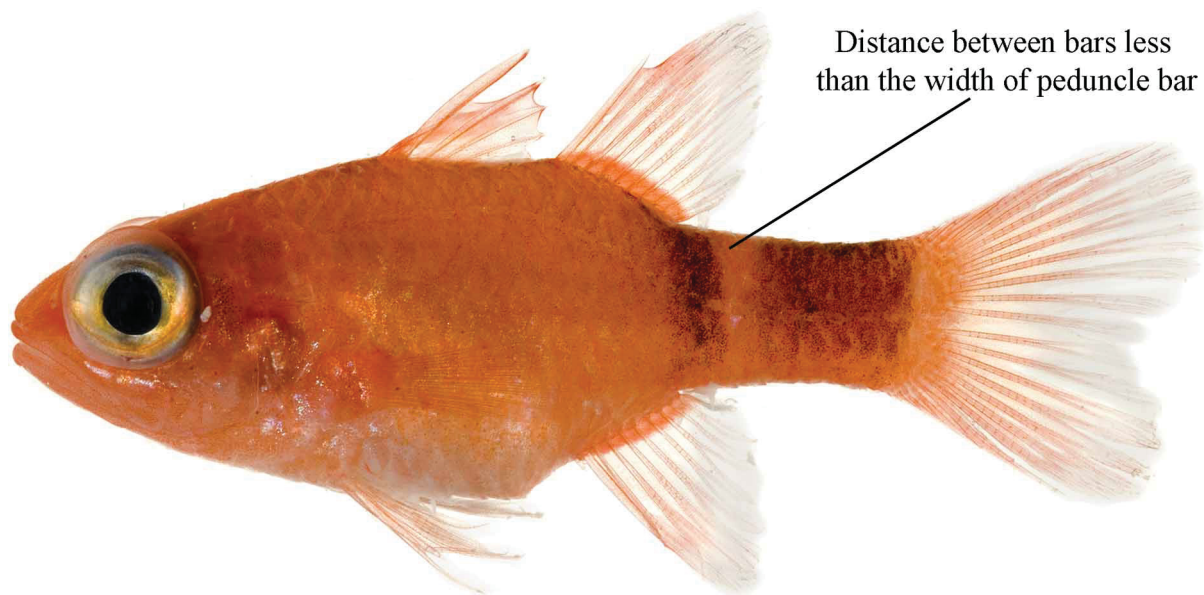
pigment on the anterior portion of the body; from *A. maculatus* in lacking prominent orange pigment on the first dorsal fin; from *A. aurolineatus* larvae in lacking orange/yellow dorsal, anal, and pelvic fins; and from *A. mosavi* larvae in lacking a distinctive pattern of chromatophores on the median fins. We were not able to reliably separate *Apogon binotatus* larvae from those of *A. townsendi* and *Apogon* sp. 1, although our *A. binotatus* larvae have more orange color on the body than *A. townsendi* larvae. However, the extent of orange coloration on the body of some *Apogon* larvae, and whether or not it is disrupted by pale areas, may change ontogenetically (see “*Apogon phenax*,” below).

Preserved larval specimens of *A. binotatus* have more melanophores on top of the head than larval *A. aurolineatus* and a longer caudal peduncle (caudal-peduncle length 33–37% SL in *A. binotatus* vs. 27–29% SL in *A. aurolineatus*). Caudal-peduncle length also is sometimes useful in separating preserved *A. binotatus* from preserved *A. planifrons* (caudal-peduncle length 33–37% SL in *A. binotatus*, 35–40% SL in *A. planifrons*), and it may be useful in separating larval *A. binotatus* from *A. maculatus* (caudal-peduncle length 30% SL in the single larval specimen of *A. maculatus* in our study material). We have identified no other features to separate preserved *A. binotatus* larvae from other known *Apogon* larvae.

### ***Apogon pillionatus* Böhlke and Randall**

**Identification.** Adult *Apogon pillionatus* (Fig. 5) is diagnosed by the combination of eight segmented anal-fin rays, the body and lateral-line scales of similar size, a dark bar just behind the second dorsal fin that does not reach the ventral midline, and a very broad bar on the posterior part of the caudal peduncle (also does not reach the ventral midline). The distance between the two body bars is considerably less than the width of the posterior bar (Böhlke & Chaplin 1993; Gon 2002). No adult specimens of *A. pillionatus* were collected in this study, and no COI sequences for the species were found in GenBank. The specimen featured in Figure 5 was collected on Saba Bank Atoll prior to our study and is not a DNA voucher specimen. Juveniles identified in this study as *A. pillionatus* (Appendix 1) have a relatively narrower bar of dark pigment on the posterior part of caudal peduncle than adult *A. pillionatus* (see “Juveniles,” below), and the anterior dark bar is situated behind the posterior base of the second dorsal fin. The identification of those juveniles as *A. pillionatus* was accomplished by process of elimination and comparative morphological examination. Six western Atlantic *Apogon* species, *Apogon planifrons*, *A. phenax*, *A. robinsi*, *A. townsendi*, *A. gouldi*, and *A. pillionatus*, have two dark bars on the posterior part of the body: one in the area of the posterior portion of the second dorsal fin and the other on the caudal peduncle (Böhlke & Randall, 1993). In *A. townsendi*, *A. planifrons*, and *A. gouldi* the anterior bar is situated entirely beneath the second dorsal-fin base. *Apogon phenax* has a wedge-shaped bar situated below and just behind the second dorsal-fin base. Only two *Apogon* species have the anterior bar well behind the end of the second dorsal fin: *A. robinsi* and *A. pillionatus*. *Apogon robinsi* is easily recognized by the lateral extensions of the premaxillary tooth patches (see “Identification” under “*A. robinsi*”), such that a portion of the dentition lies outside the confines of the mouth (Böhlke & Randall, 1968; Böhlke & Chaplin 1993; Gon 2002). Furthermore, the anterior body bar in *A. robinsi* extends to the ventral midline, whereas in *A. pillionatus* it falls short of the ventral midline. The juveniles identified herein as *A. pillionatus* (Fig. 1) have the anterior bar well behind the second dorsal-fin base, that bar terminating well short of the ventral midline, and no dentition outside of the mouth.

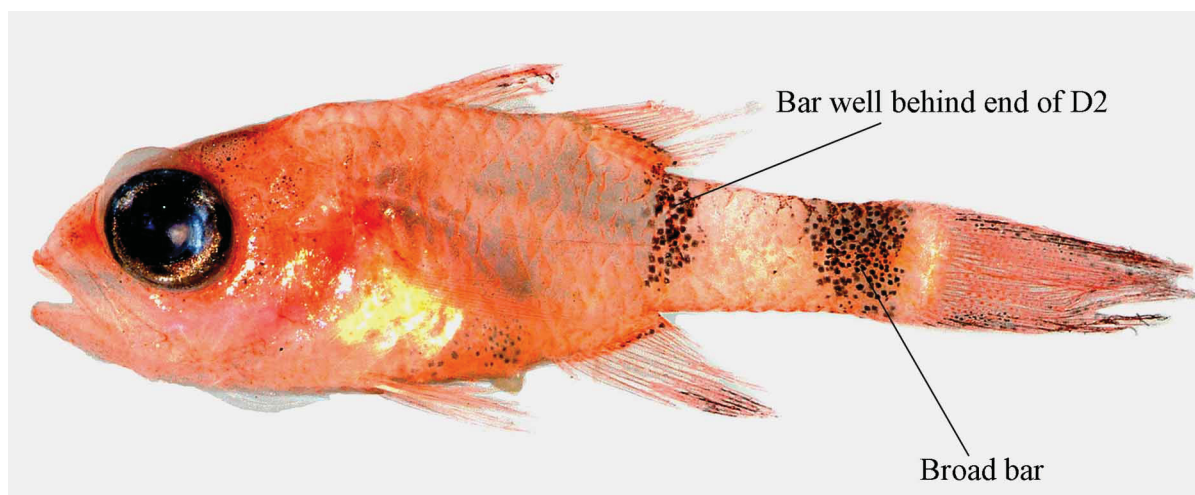




**FIGURE 5.** *Apogon pillionatus*, adult, 36.0 mm SL, SABA 06–021, not a DNA voucher specimen. Photograph by J. T. Williams.

**Juveniles (Fig. 6).** Five juveniles identified as described above range from 15.0 to 17.0 mm SL. The body is pale orange with darker orange coloration on the head and anterior rays of the first dorsal fin. There are melanophores on top of head, behind the eye on the cheek and temporal regions, and on the gut. There are melanophores on the posterior part of the second dorsal- and anal-fin bases, as well as on the distal tips of the middle rays of the first dorsal and anal fins. There are numerous melanophores on the outer rays of the caudal fin. The anterior bar of the body is entirely behind the posterior end of the second dorsal-fin base and does not reach the ventral midline of the body. The bar on the caudal peduncle is broad. As noted above, this bar is narrower in juveniles than in adults, and the space between the anterior and posterior body bars in juveniles is equal to or greater than the width of the posterior bar (this space smaller in adults).

**Comparisons Among Juveniles.** Characters used to separate juvenile *A. pillionatus* from other *Apogon* species having two body bars are discussed above (see “Identification”).



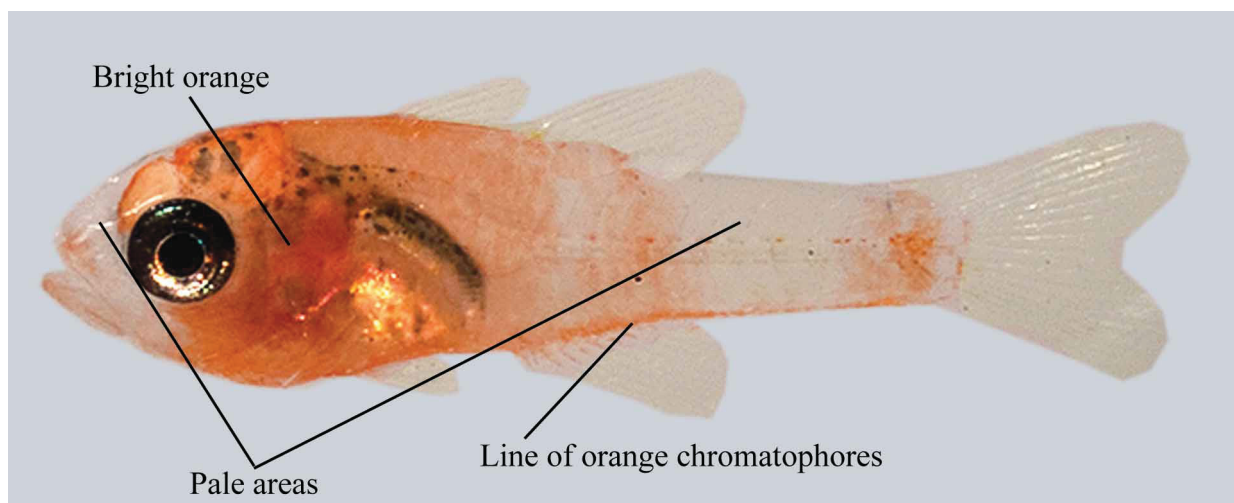
**FIGURE 6.** *Apogon pillionatus*, juvenile, 16.0 mm SL, DNA # BLZ 8112, reared, photograph by C. Baldwin and L. Weigt.

## *Apogon* sp. 1

**Identification.** No adults match the single larval specimen of this lineage in our genetic analysis. This unidentified species could be *A. evermanni*, *A. leptocaulus*, or *A. gouldi*, and specimens of those species are needed for comparative genetic analysis. Alternatively, the unidentified larval specimen may represent an undescribed species.

**Larvae (Fig. 7).** *Apogon* sp. 1 is represented by one 10.0 mm SL specimen. The body is mostly orange with some transparent areas. The snout is mostly transparent, but there is a small, pale orange spot at the anterior tip. The jaws are pale orange. The rest of the head and anterior portion of the trunk are orange, the area immediately behind the eye the most intense orange. The posterior region of the body is mostly transparent, with one band of pale orange pigment below the second dorsal fin and one spot of bright orange pigment on caudal peduncle. There is a line of bright orange pigment on the ventral midline of the body from the anal fin to the caudal peduncle. All fins are clear except the proximal portions of the anal-fin rays have pale orange coloration. There are melanophores on top of the head, behind the eye on the temporal region, over the swimbladder, and on the lateral surface of the gut. The caudal-peduncle length is 35% SL.

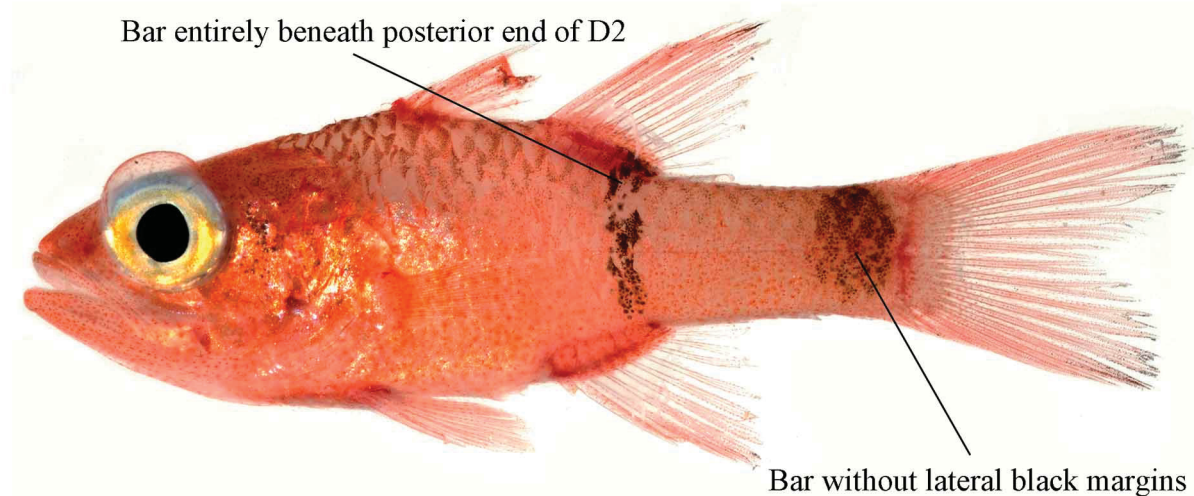
**Comparisons Among Larvae.** Larval *Apogon* sp. 1 most closely resemble *A. binotatus*, *A. phenax*, *A. planifrons*, and *A. townsendi* larvae in having orange pigmentation concentrated mainly on the head and anterior portion of the trunk and transparent areas on the snout and usually also on posterior portions of the trunk. *Apogon* sp. 1 differs from *A. planifrons* in lacking yellow pigment on the anterior portion of the body and from larval *A. phenax* in lacking a prominent orange spot on the snout. From *A. maculatus*, larval *Apogon* sp. 1 differs in lacking orange pigment on the first dorsal fin and in having a longer caudal peduncle (35% SL in *Apogon* sp. 1, 30% SL in *A. maculatus*). From *A. aurolineatus*, larval *Apogon* sp. 1 differs in lacking orange/yellow pigment on the dorsal, pelvic, and anal fins; in having transparent areas on the trunk (vs. trunk completely orange in *A. aurolineatus*); and in having a longer caudal peduncle (35% SL in *Apogon* sp. 1 vs. 27–29% SL in *A. aurolineatus*). *Apogon* sp. 1 lacks the distinctive pattern of chromatophores on the median fins typical of *A. mosavi* and the single specimen examined has a longer caudal peduncle (35% SL in *Apogon* sp. 1 vs. 31–34% SL in *A. mosavi*). Additional larval specimens of *Apogon* sp. 1 are needed to confirm the differences noted above and to determine if it can be separated from larval *A. binotatus* and *A. townsendi*.



**FIGURE 7.** *Apogon* sp. 1, larva, 10.0 mm SL, DNA # BLZ 5260, photograph by J. Mounts.

## *Apogon planifrons* Longley and Hildebrand

**Identification.** One adult specimen of *A. planifrons* (Fig. 8) provided the basis for genetic identification of five larva (Appendix 1). No juveniles are present in our material. Adult *A. planifrons* has eight segmented anal-fin rays, the body and lateral-line scales of similar size, the anterior dark bar positioned entirely below the posterior end of the second dorsal fin and distinctly narrower than the dark bar on the caudal peduncle, 15 or 16 circum-caudal-peduncle scales, and usually 15 (14–16) gill rakers on the lower limb of the first arch (Böhlke & Chaplin 1993, Böhlke & Randall 1968, Gon 2002). *Apogon planifrons* most closely resembles *A. townsendi* and *A. gouldi* in having the anterior bar entirely beneath the second dorsal-fin base. It can be separated from those species by lower-limb rakers (Table 2) and circum-caudal-peduncle scales (15–16 in *A. planifrons* vs. 12 in *A. townsendi* and *A. gouldi*). *Apogon planifrons* can be further distinguished from *A. townsendi* in lacking black lateral margins on the caudal-peduncular bar (Böhlke & Chaplin 1993, Smith-Vaniz 1977, Gon 2002).



**FIGURE 8.** *Apogon planifrons*, adult, 37.0 mm SL, DNA # BLZ 5270, photograph by J. Mounts.

**Larvae (Fig. 9).** *Apogon planifrons* larvae genetically analyzed in this study range from 9.0 to 10.0 mm SL. The snout is mostly transparent, and there is pale orange coloration on the jaws. The central and posterior portions of the head and the belly have prominent yellow pigmentation. The posterior region of the body is mostly pale orange to orange in 9.0 and 9.5 mm SL specimens, with some pale areas on the caudal peduncle. In 10-mm SL specimens, much of the posterior portion of the body is pale, usually with an orange bar beneath the posterior end of the second dorsal fin and an orange blotch on the posterior end of the caudal peduncle. The dorsal fins are clear. There are orange chromatophores at the bases of the pelvic and anal fins, as well as on the proximal portion of the ventral lobe of the caudal fin. There is a line of orange chromatophores along the anal-fin base that extends onto the ventral midline of the caudal peduncle. There are melanophores on top of the head, behind eye in the temporal region, over the swimbladder, and on the lateral surface of the gut. The caudal peduncle is long, 35–40% SL.

**Comparisons Among Larvae.** *Apogon planifrons* larvae are easily distinguished from other known *Apogon* larvae by the bright yellow chromatophores on most of the head and abdominal region and usually by a long caudal peduncle (35–40% SL in *A. planifrons* vs. 27–37% SL in other *Apogon* species). Caudal-peduncle length alone is useful for separating preserved larval specimens of *A. planifrons* from larvae of some *Apogon* species, but *A. phenax*, *A. binotatus*, and *A. townsendi* also have a long caudal peduncle (32–37% SL). We know of no features that distinguish preserved larvae of *A. planifrons* from those species.



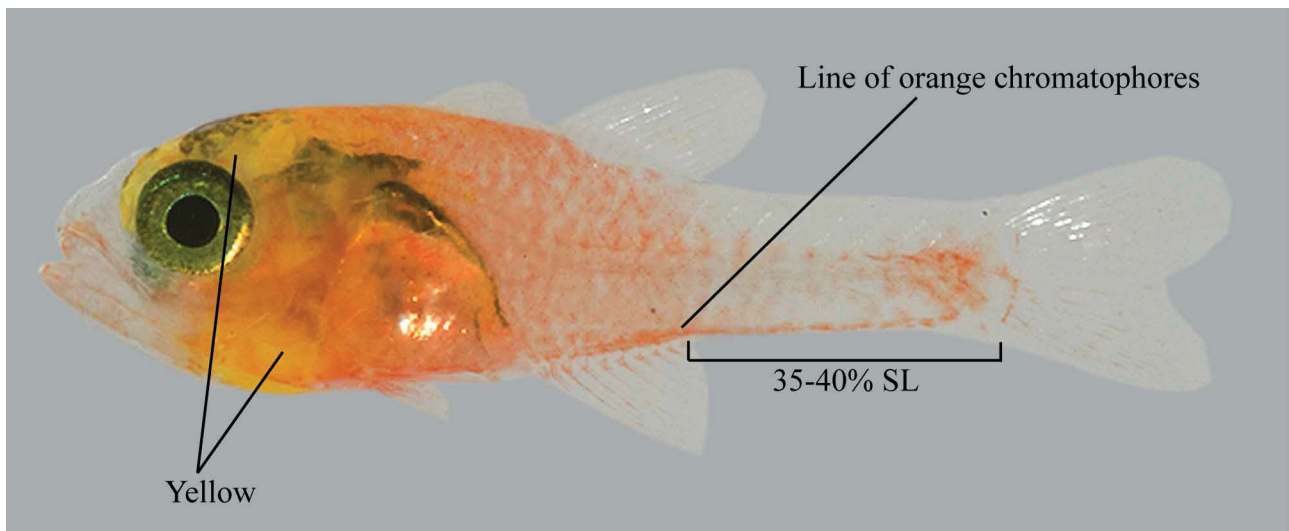


FIGURE 9. *Apogon planifrons*, larva, 9.5 mm SL, DNA # BLZ 7126, photograph by J. Mounts.

### *Apogon phenax* Böhlke and Randall

**Identification.** Sixteen adult specimens of *A. phenax* provided the basis for genetic identification of larvae and juveniles (Appendix 1, one adult is shown in Fig. 10). Adult *A. phenax* can be distinguished from other *Apogon* by the combination of eight segmented anal-fin rays, body and lateral-line scales of similar size, body with two distinct dark markings (one wedge-shaped bar below and just behind second dorsal fin and a bar on the posterior part of caudal peduncle—the distance between the two bars larger than the width of the posterior bar), and 11 to 14 (usually 13–14) gill rakers (Böhlke & Chaplin 1993, Gon 2002).

**Juveniles (Fig. 11).** Eight juveniles, 16.0–22.0 mm SL, are present in our material. The body is pale orange. The head, abdomen, first dorsal fin, bases of second dorsal and anal fins, and posterior portion of the caudal peduncle are darker pink/orange. There are melanophores on the head, gut, outer rays of the caudal fin and on the distal portions of the second dorsal and anal fins. Two dark bars are present on the trunk in all juveniles. The anterior bar is somewhat wedge shaped (slightly broader dorsally than ventrally) and extends ventrally to a point slightly below mid body or slightly above the anal-fin base. This bar is confluent with a stripe of melanophores along the second dorsal-fin base and separated by a gap from a similar stripe along the anal-fin base. The bar on the caudal peduncle extends from the dorsal to the ventral margins of the body. As in adults, the distance between the two bars is greater than the width of the posterior bar. There are 13–14 lower-limb gill rakers on the first arch in the juveniles, which is consistent with values for adults (Table 2).

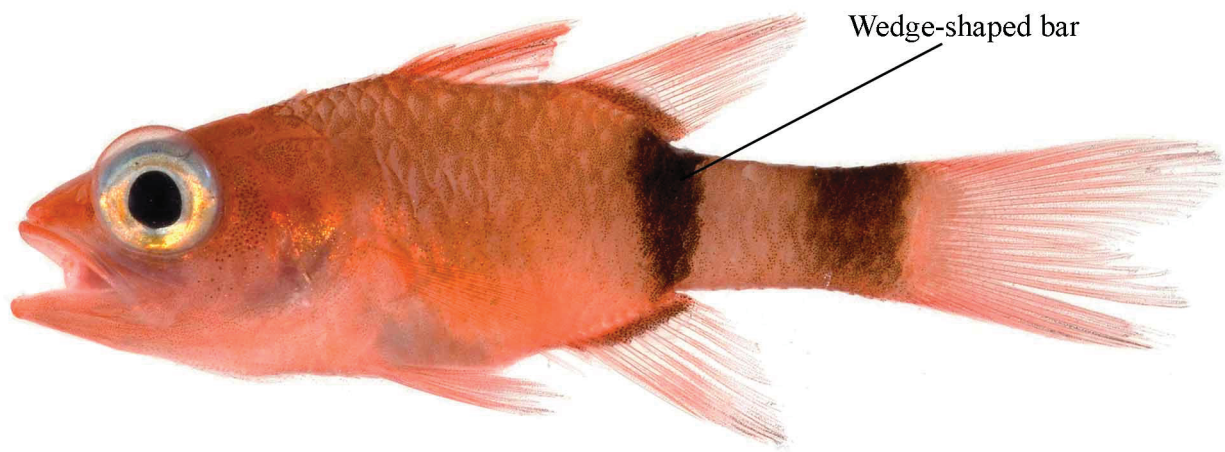


FIGURE 10. *Apogon phenax*, adult, 32.0 mm SL, DNA # BLZ 5268, photograph by J. Mounts.

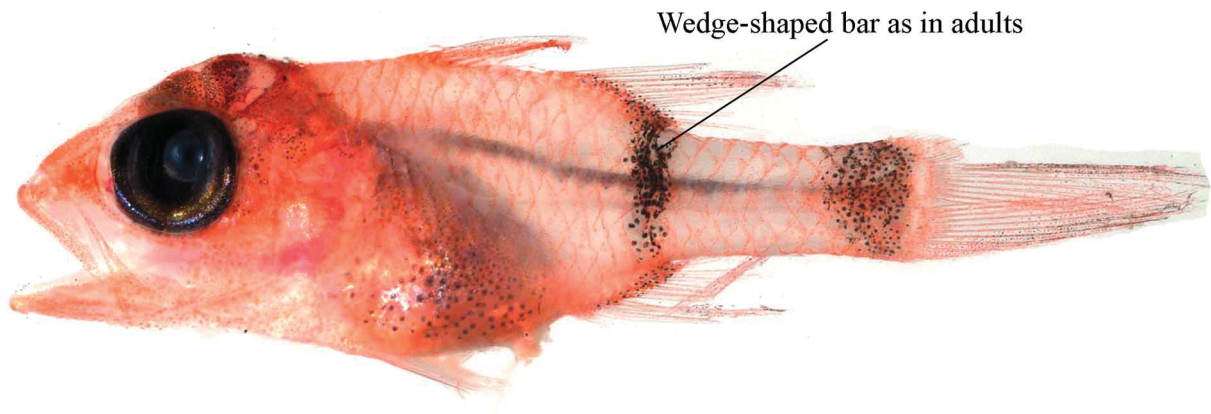


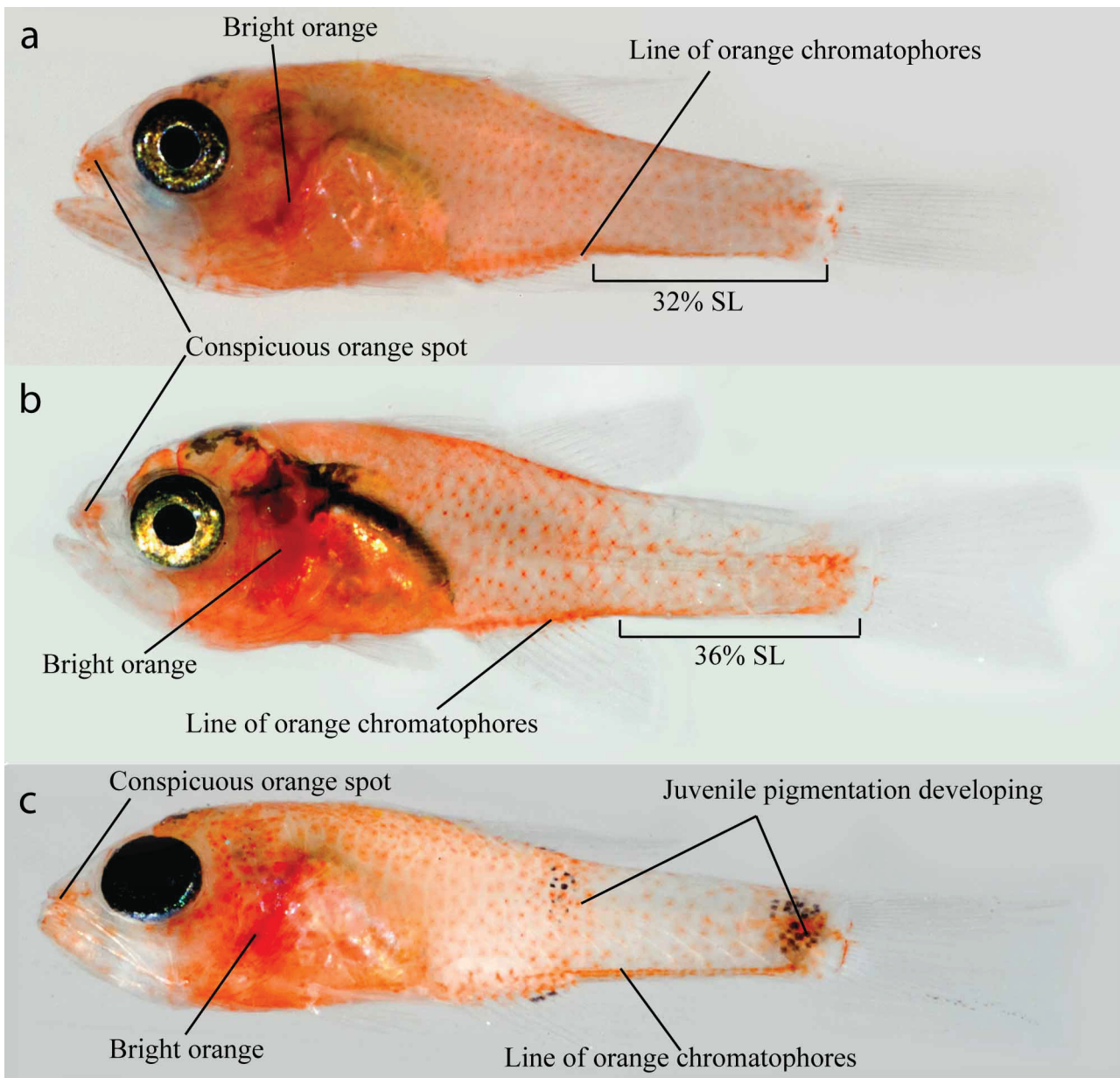
FIGURE 11. *Apogon phenax*, juvenile, 16.0 mm SL, DNA # BLZ 8166, photograph by C. Baldwin and L. Weigt.

**Comparisons Among Juveniles.** Juvenile *A. phenax* can be distinguished from other juvenile *Apogon* by the same characters that separate adults.

**Larvae (Fig. 12).** *Apogon phenax* larvae genetically analyzed in this study range from 9.5 to 11.0 mm SL. The smaller specimens are bright orange in life, and the fins are clear (Fig. 12a). Much of the snout is transparent, but there is a conspicuous orange spot above the upper lip. The upper and lower jaws have scattered orange chromatophores. A nearly solid line of orange pigment extends along the base of the anal fin and ventral midline of the caudal peduncle. A 10.0-mm SL specimen (Fig. 12b) is paler orange in general, has more pale areas on the snout and jaws, and has a large pale area on the dorsal portion of the caudal peduncle. There are a few orange chromatophores at the bases of the central and ventral rays of the caudal fin. In one 11.0 mm SL specimen (Fig. 12c), the dark bars characteristic of juveniles and adults are beginning to form posteriorly, and the area between the bars is pale. In all larvae there are melanophores on top of the head, in the temporal region, and over the swim bladder and gut. The caudal-peduncle length ranges from 32–36% SL.

**Comparisons Among Larvae.** The smallest larvae of *A. phenax* resemble larvae of *A. aurolineatus* and *A. maculatus* in usually having a bright orange body color, but they differ in lacking orange/yellow (*A. aurolineatus*) or orange (*A. maculatus*) pigment on the first dorsal fin; *A. aurolineatus* also has orange second dorsal, anal, and pelvic fins, which are clear in *A. phenax* larvae. Additionally, larvae of *A. phenax* have more melanophores on top of the head than *A. aurolineatus* and a longer caudal peduncle than that species and *A. maculatus* (caudal-peduncle length 32% SL or larger in *A. phenax*, 27–29% SL in *A. aurolineatus*, 30% in *A. maculatus*). Larger *Apogon phenax* larvae are very similar to those of *A. binotatus*, and somewhat similar to those of *A. townsendi*, but they differ from both in having a conspicuous orange spot above the upper lip (vs. small and pale, if present). Larval *A. phenax* can be separated from larval *A. planifrons* by the absence of bright yellow pigment on the head and from larval *A. mosavi* by the absence of a distinctive pattern of chromatophores on the median fins. We have identified no morphological features to separate preserved *A. phenax*, *A. binotatus*, *A. townsendi*, and *A. planifrons* larvae.

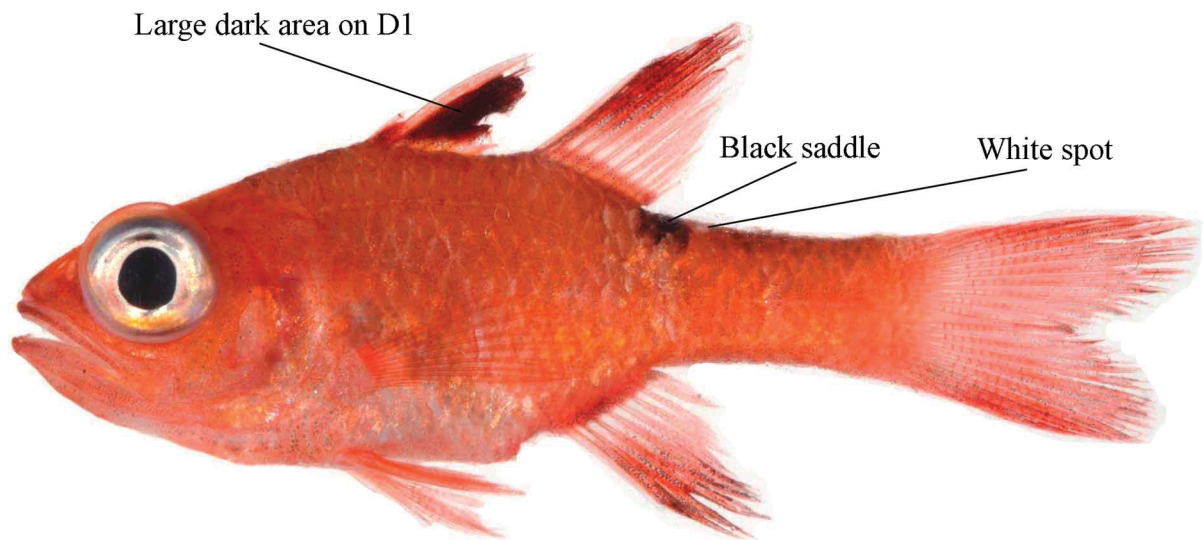




**FIGURE 12.** *Apogon phenax* a) larva, 9.5 mm SL, DNA # BLZ 6335; b) larva, 10.0 mm SL, DNA # BLZ 6361; c) larva, 11.0 mm SL, DNA # BLZ 6359; photographs by J. Mounts and C. Baldwin.

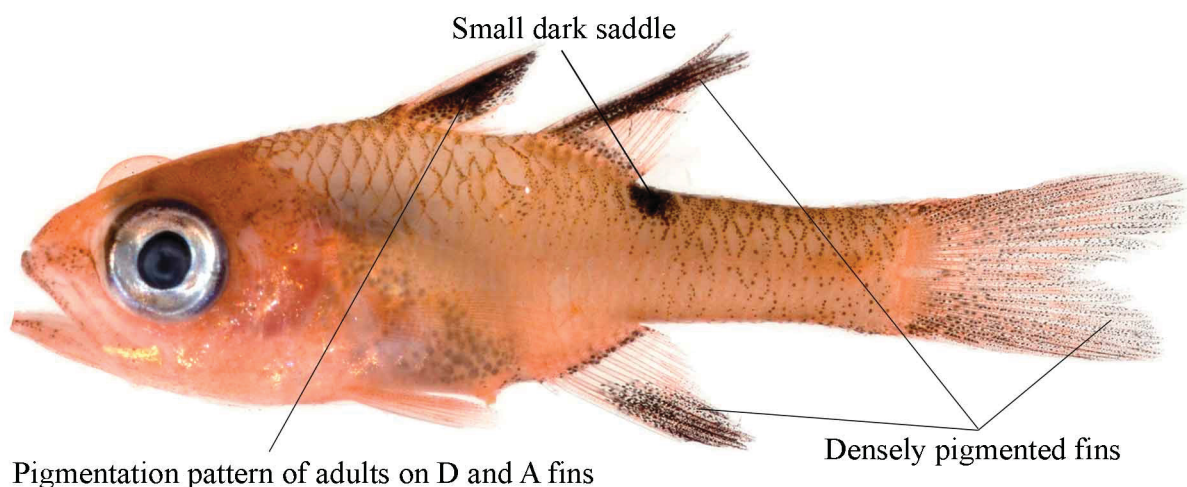
### *Apogon lachneri* Böhlke

**Identification.** Four adult specimens of *A. lachneri* provided the basis for genetic identification of seven juveniles (Appendix 1; one adult is shown in Fig. 13). The combination of characters that distinguishes *A. lachneri* adults from other *Apogon* species is eight segmented anal-fin rays; 16–17 gill rakers on the lower limb of the first arch; lateral-line and body scales of similar size; a small dark saddle behind the second dorsal fin, followed by a white spot (white spot may not be apparent in preserved specimens); a large dark area on the first dorsal fin posterior to the second spine; and anterior portions of the second dorsal and anal fins dark to dusky distally (Böhlke & Chaplin 1993, Gon 2002).



**FIGURE 13.** *Apogon lachneri*, adult, 36.0 mm SL, DNA # BLZ 5118, photograph by J. Mounts and C. Baldwin.

**Juveniles (Fig. 14).** Juveniles examined range from 18.0–22.0 mm SL. In all specimens, the body is pale orange, and the dark pattern of pigment on the dorsal and anal fins typical of adults is conspicuous. The caudal-fin rays are densely covered with melanophores. There are scattered melanophores on the head in the smallest juveniles. The posterior margins of the scales on the dorsal portion of the trunk and caudal peduncle are covered with melanophores, forming roughly diamond-shaped patterns of pigment on the body. The dark saddle behind the second dorsal fin characteristic of adults is beginning to develop or fully present in all specimens. There are 16 gill rakers on the lower limb of the first gill arch.

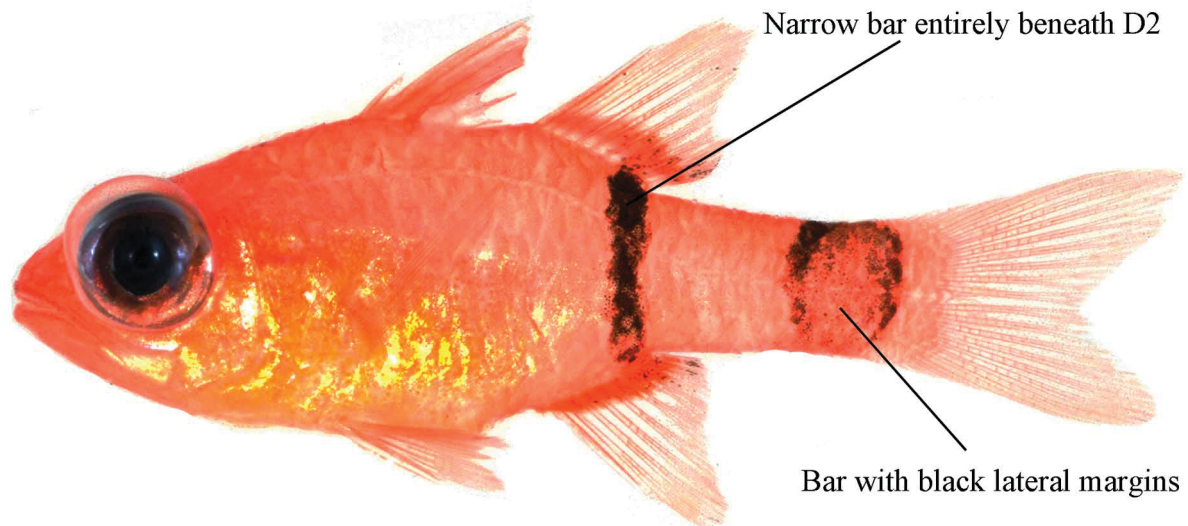


**FIGURE 14.** *Apogon lachneri*, juvenile, 21.0 mm SL, DNA # BLZ 5265, photograph by J. Mounts.

**Comparisons Among Juveniles.** Juvenile *A. lachneri* can be distinguished from all *Apogon* juveniles by the conspicuous dark pigment on the dorsal and anal fins. It resembles *A. aurolineatus* in lacking dark markings on the caudal peduncle, but it is easily separated from that species by the dark saddle behind the second dorsal fin (vs. no distinguishing marks on the body in juvenile *A. aurolineatus*). *Apogon lachneri* juveniles most closely resemble those of *A. maculatus* and *A. pseudomaculatus* in having a dark spot of pigment associated with the base of the second dorsal fin, but the position of the spot distinguishes them (behind the last ray of the second dorsal fin in *A. lachneri*; on the posterior base of that fin in *A. maculatus*, and well below the posterior base of the second dorsal fin in *A. pseudomaculatus*). Juvenile *A. lachneri* can further be distinguished from *A. maculatus* and *A. pseudomaculatus* by lacking a dark blotch of pigment on the caudal peduncle.

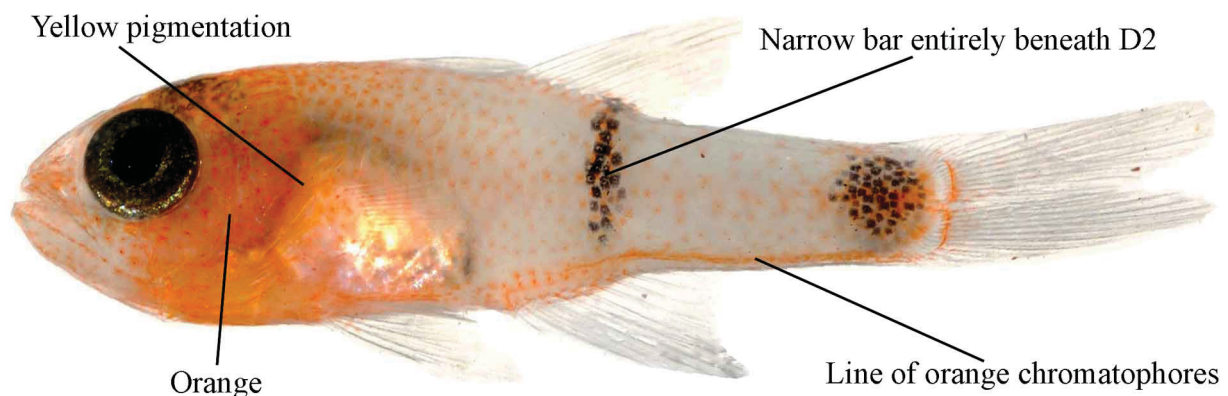
*Apogon townsendi* (Breder)

**Identification.** Twelve adult specimens of *A. townsendi* provided the basis for genetic identification of larvae and juveniles (Appendix 1, one adult is shown in Fig. 15). Adult *A. townsendi* can be distinguished from other *Apogon* by the combination of eight segmented anal-fin rays, body and lateral-line scales of similar size, the anterior body bar narrow and entirely beneath the second dorsal fin, the posterior body bar with black lateral margins, 12 circum-caudal-peduncle scales, and 17 gill rakers on the lower limb of first gill arch (Böhlke & Chaplin 1993, Gon 2002).



**FIGURE 15.** *Apogon townsendi*, adult, 34.0 mm SL, DNA # BLZ 7833, photograph by C. Baldwin and L. Weigt.

**Juveniles (Fig. 16).** Thirteen juveniles of *A. townsendi* (12.0 to 21.0 mm SL) were identified in our material (Appendix 1). The body is pale orange with more intense orange and some yellow coloration on the head. There is a line of distinctive orange pigment extending along the ventral portion of the body from the base of the pelvic fin posteriorly to the base of the caudal fin. Orange chromatophores are mixed with melanophores in the bar of pigment beneath the second dorsal fin and in the blotch of pigment on the caudal peduncle. The fins are mostly clear, but there are usually a few chromatophores at the bases of the anterior rays of the first dorsal fin and on the bases of the pelvic and anal fins. There are two roughly vertical lines of orange at the base of the caudal fin, one on the upper lobe and one on the lower. There are melanophores on top of the head and internally above the swimbladder and gut. Both body bars typical of adults are present, but the peduncular bar lacks the diagnostic dark lateral margins. The juveniles have 16–18 gill rakers on the lower limb of first gill arch.

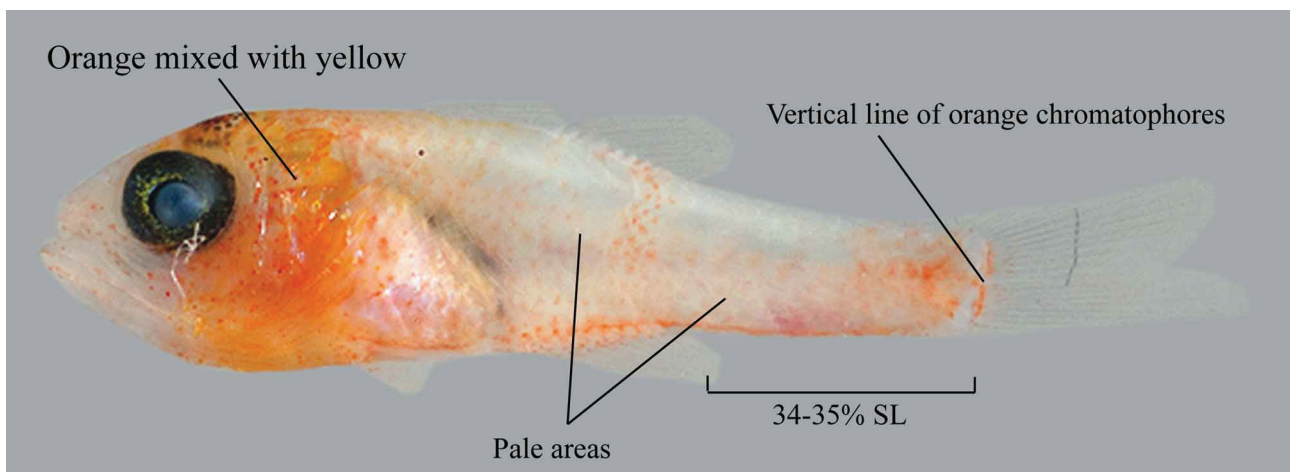


**FIGURE 16.** *Apogon townsendi*, juvenile, 13.0 mm SL, DNA # BLZ 4542, photograph by L. Weigt.



**Comparisons Among Juveniles.** The absence of dark lateral margins on the dark bar on the caudal peduncle in juvenile *A. townsendi* could result in confusing this species with juveniles of *A. pillionatus*, *A. phenax*, and, presumably, *A. robinsi* and *A. planifrons* (juveniles of the last two species not present in our material). It can be separated from juvenile *A. pillionatus* and *A. phenax* in having at least some yellow coloration on the head (vs. pale orange in *A. pillionatus* and *A. phenax*). It can also be separated from those species, and presumably *A. robinsi*, by having the anterior bar entirely beneath the second dorsal fin (vs. behind the second-dorsal fin in *A. pillionatus* and *A. robinsi*; beneath and just behind the second-dorsal fin in *A. phenax*). Additionally, there are no teeth on the lateral surface of the premaxilla in *A. townsendi* as there are in *A. robinsi*. It seems likely that juvenile *A. planifrons* will exhibit some yellow coloration on the head and may be difficult to distinguish from juvenile *A. townsendi*. Both species have the anterior dorsal bar entirely beneath the second dorsal fin. In the absence of the diagnostic dark lateral margins on the peduncular bar in juvenile *A. townsendi*, gill rakers are the best way to separate juveniles of the two species: there are usually 17 (16–18) lower-limb rakers in *A. townsendi*, and usually 15 (14–16) in *A. planifrons* (Böhlke & Chaplin 1993, Gon 2002; Table 2).

**Larvae (Fig. 17).** The two *Apogon townsendi* larvae genetically analyzed in this study are 11.0 mm SL. In both specimens much of the snout is transparent, but the anterior portion of the snout and the jaws are pale orange. The rest of the head is darker orange, and there appear to be some yellow chromatophores mixed in. Posterior to the head there are extensive pale areas on the body, an orange bar beneath the posterior end of the second dorsal fin, and a darker orange blotch on the posterior end of the caudal peduncle. There is a line of orange pigment on the ventral portion of the body from the base of the pelvic fin to the base of the caudal fin. The fins are mostly clear, but there are a few orange chromatophores on the bases of the pelvic and anal fins. There are two roughly vertical lines of orange on the caudal-fin base, one on the upper lobe and one on the lower. There are melanophores on top of the head and internally over the swimbladder and gut. The caudal peduncle length is 34–35% SL.

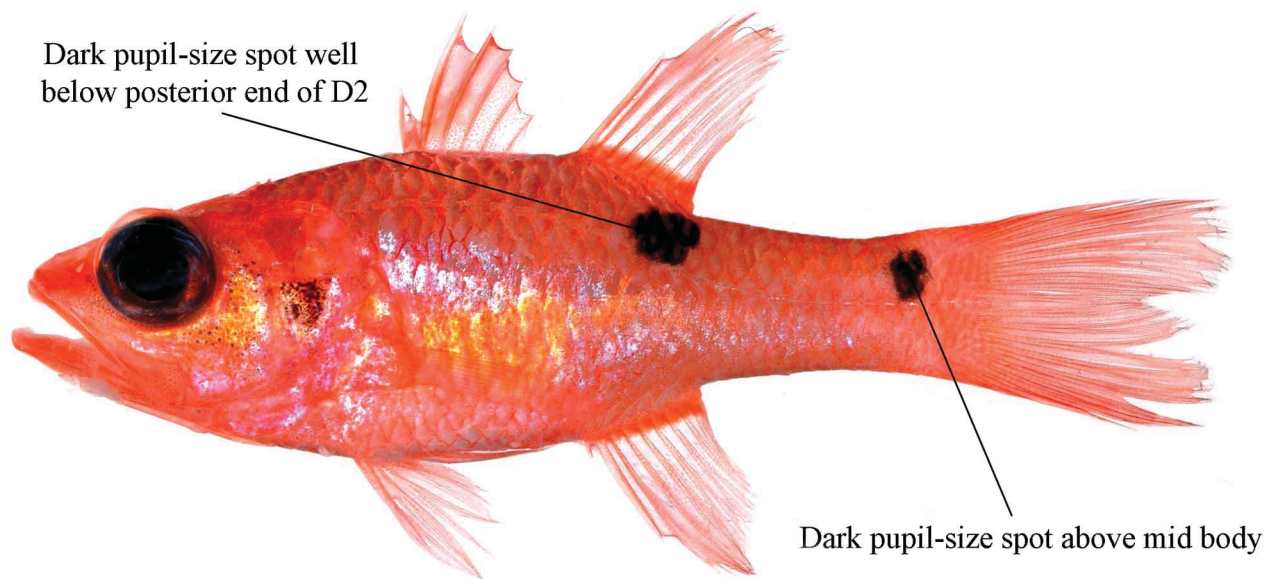


**FIGURE 17.** *Apogon townsendi*, larva, 11.0 mm SL, DNA # BLZ 6329, photograph by J. Mounts and C. Baldwin.

**Comparisons Among Larvae.** *Apogon townsendi* larvae are similar to those of *A. planifrons*, *A. binotatus*, *A. phenax*, and *Apogon* sp. 1 in the pattern of chromatophores on the body. They are most easily distinguished from *A. planifrons* in having primarily orange vs. yellow chromatophores on the head. Larval *A. townsendi* differs from *A. phenax* in lacking a prominent orange spot on the snout. From *A. binotatus* and *Apogon* sp. 1, larval *A. townsendi* may differ in having more prominent orange pigment on the caudal-fin base—in two roughly vertical lines, but there is considerable variation in pigment in this region among larval *Apogon*. From *A. maculatus* and *A. aurolineatus*, larval *A. townsendi* differs in lacking orange or yellow pigment on the first dorsal fin and in having a longer caudal peduncle (peduncle length 34–35% SL in *A. townsendi*, 30% in *A. maculatus*, 27–29% in *A. aurolineatus*). Caudal-peduncle length also is useful in separating preserved specimens of those species, and preserved larval *A. townsendi* also have more melanophores on the top of the head than *A. aurolineatus*.

## *Apogon pseudomaculatus* Longley

**Identification.** One wild-caught adult specimen of *A. pseudomaculatus* from Florida provided the basis for genetic identification of one juvenile reared from a wild-caught larva from Belize and one juvenile specimen from Curaçao (Appendix 1). An adult collected off Curaçao but not yet analyzed genetically is shown in Figure 18. The combination of characters that distinguishes *A. pseudomaculatus* adults from other *Apogon* species is the presence of eight segmented anal-fin rays, body and lateral-line scales of similar size, dark pupil-size spot below posterior end of second dorsal fin, 14–16 circum-caudal-peduncle scales, and a dark pupil-size spot on the caudal peduncle (Böhlke & Chaplin 1993; Gon 2002). A color image of FWRI 20646, the genetically analyzed adult (quality of specimen and image too poor to reproduce here), shows the dark spot beneath the second dorsal fin and another on the caudal peduncle. The spot beneath the second dorsal fin is well below the base of the fin, a diagnostic feature of *A. pseudomaculatus*. However, there is black pigment on the dorsal, caudal, and anal fins on the FWRI specimen that is not present in the adult specimen from Curaçao (Fig. 18). Further comparative study, including genetic analysis of the Curaçao specimen, is needed.



**FIGURE 18.** *Apogon pseudomaculatus*, adult, 60.0 mm SL, DNA # CUR 11003, photograph by C. Castillo and C. Baldwin.

**Juveniles (Fig. 19).** The two juveniles are pale to bright orange. Most fins have some orange coloration, and the first dorsal is predominantly orange. There are melanophores on the anterior rays of the first dorsal, second dorsal, and anal fins, as well as on the anterior base of the second dorsal fin. The outer rays of the caudal fin are densely pigmented. There is a dark spot behind the eye on the opercle and two white stripes in the eye, one above and one below the pupil. There are two dark spots on the body, one on the trunk well below the posterior base of the second dorsal fin and one on the caudal peduncle. The latter is mostly situated above the lateral line and tapers ventrally. There are 13–14 gill rakers on the lower limb of the first gill arch.

**Comparisons Among Juveniles.** Juveniles of *A. pseudomaculatus* most closely resemble juvenile *A. maculatus* and *A. lachneri* in having a spot or blotch of pigment beneath the second dorsal fin (vs. bars of pigment in juveniles of *A. binotatus*, *A. pillionatus*, *A. phenax* and *A. townsendi*, and no pigment beneath the second dorsal fin in *A. aurolineatus*). Juvenile *A. pseudomaculatus* differs from juvenile *A. lachneri* in having the trunk blotch positioned well below the second dorsal-fin base (vs. just behind the second dorsal-fin base) and in having a dark blotch on the caudal peduncle (lacking in *A. lachneri*). *Apogon pseudomaculatus* juveniles can be distinguished from *A. maculatus* juveniles by the position of the spot beneath the second dorsal fin (well below it in *A. pseudomaculatus*, on the fin base in *A. maculatus*), and by the shape of the caudal-peduncle mark (mostly concentrated above the lateral line in *A. pseudomaculatus*, extending well below the lateral midline in *A. maculatus*).



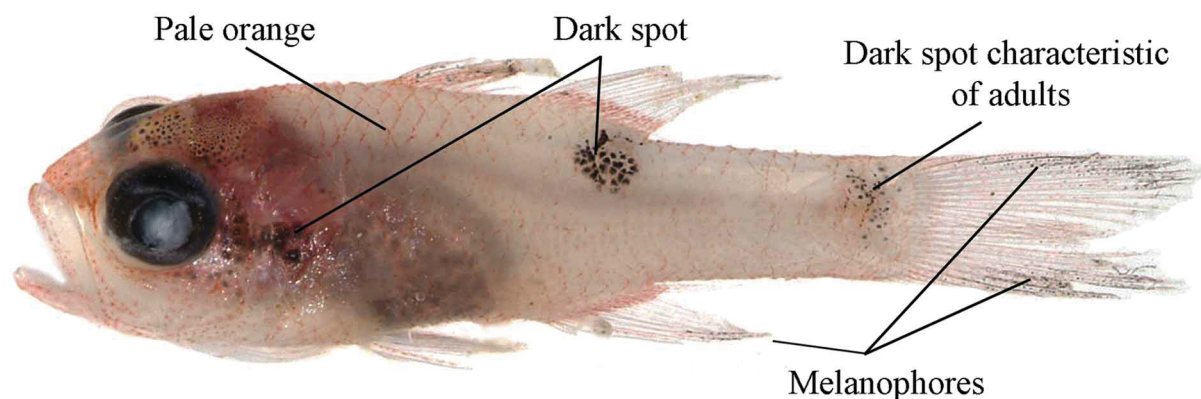


FIGURE 19. *Apogon pseudomaculatus*, juvenile, 19.0 mm SL, DNA # CUR 8079, photograph by C. Baldwin.

### *Apogon affinis* (Poey)

**Identification.** Adult *A. affinis* can be distinguished from other *Apogon* species by the combination of cycloid to weakly ctenoid scales, median predorsal scales present, pectoral-fin soft rays 11 or 12 (rarely 13), nine segmented anal-fin rays, and both jaws with a single series of small conical teeth interspersed with several enlarged caniniform teeth (Böhlke & Chaplin 1993, Gon 2002). Two adult specimens identified as *A. affinis* based on those features were analyzed genetically (Appendix 1). The specimens were taken in trawls and are not in good shape, and we selected a photograph of an adult not analyzed genetically in this study to illustrate the species (Fig. 20). No larvae or juveniles analyzed in this study genetically match *A. affinis*.

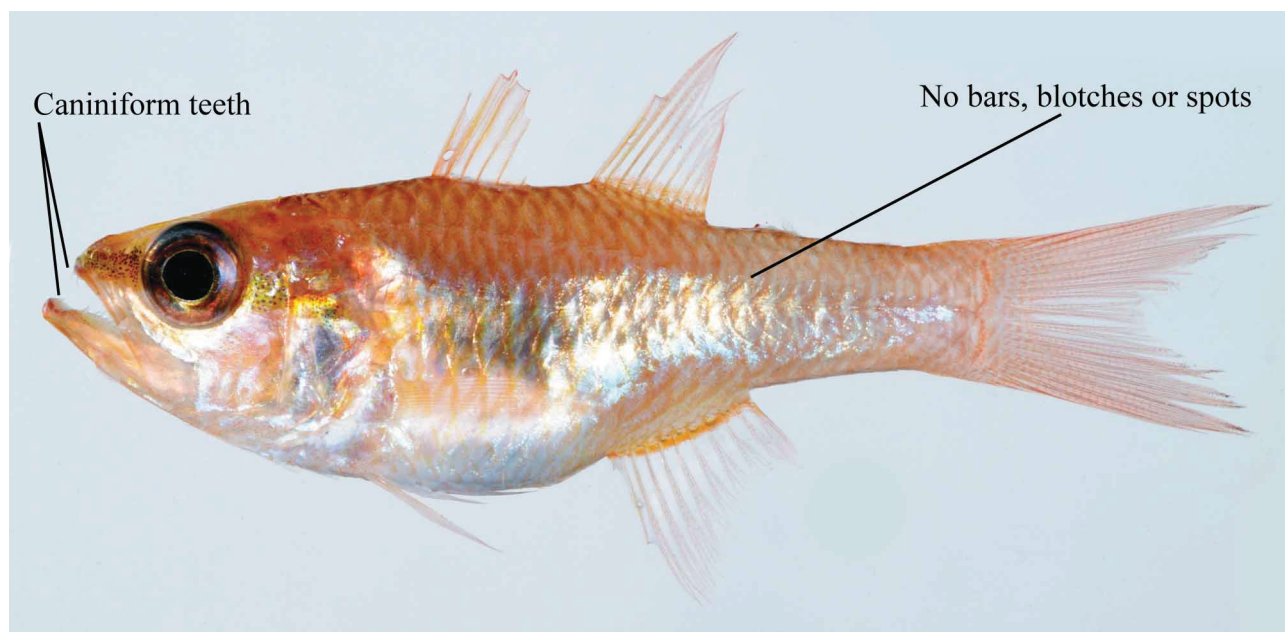


FIGURE 20. *Apogon affinis*, adult, 68.0 mm SL, DNA # CUR 11005, photograph by C. Castillo and C. Baldwin.

### *Apogon maculatus* (Poey)

**Identification.** Fifteen adult specimens of *A. maculatus* provided the basis for genetic identification of one larva and three juveniles (Appendix 1, one adult is shown in Fig. 21). The combination of characters that distinguishes *A. maculatus* adults from other *Apogon* species is eight segmented anal-fin rays, lateral-line and body scales of similar size, a dark pupil-size spot present below the posterior end of the second dorsal fin, 17–20 circum-caudal-peduncle

scales, and a large, dark caudal blotch that extends ventrally well below the lateral midline (Böhlke & Chaplin 1993; Gon 2002). One adult specimen from Belize, BLZ 5023, is more divergent in COI from other specimens of *A. maculatus* than is typical within the genus (Fig. 1), but the specimen does not appear remarkably different morphologically. In the combined data set (see summary data in Table 1), average intraspecific variation in *A. maculatus* is 1%, whereas in most other *Apogon* species it is 0%. *Apogon pillionatus* and *A. aurolineatus* also are characterized by 1% average intraspecific variation.

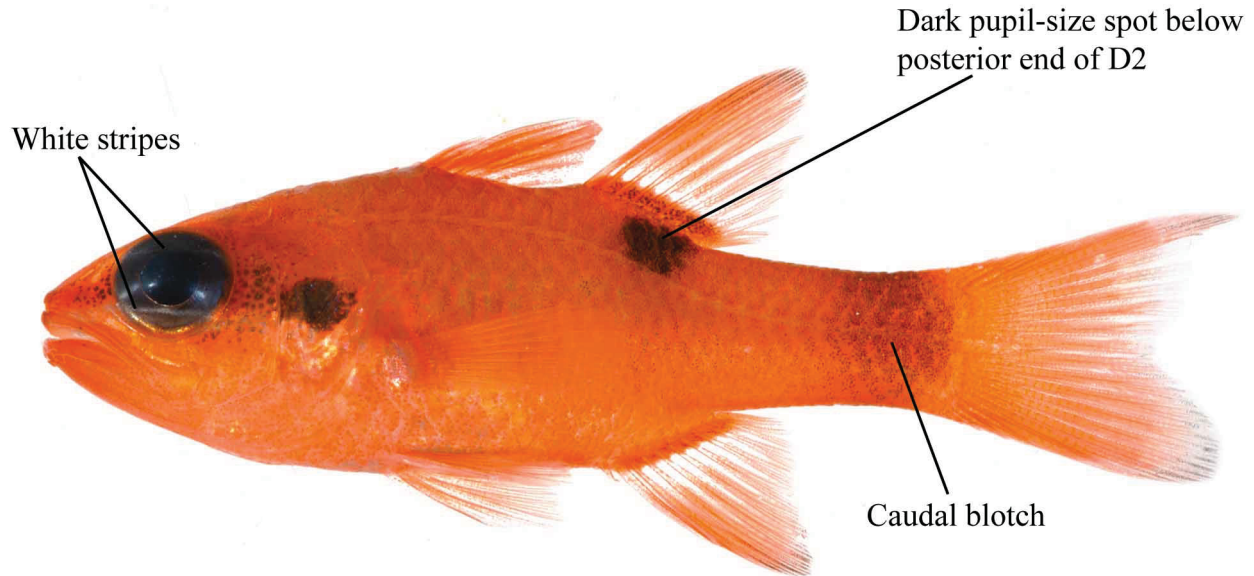


FIGURE 21. *Apogon maculatus*, adult, 38.0 mm SL, DNA # BLZ 4170, photograph by L. Weigt.

**Juveniles (Fig. 22).** The three juveniles are 16.0–22.0 mm SL. All have the adult pattern of pigmentation except that the caudal-peduncle blotch is not fully developed in all specimens. The juveniles have 13–14 gill rakers on the lower limb of first gill arch.

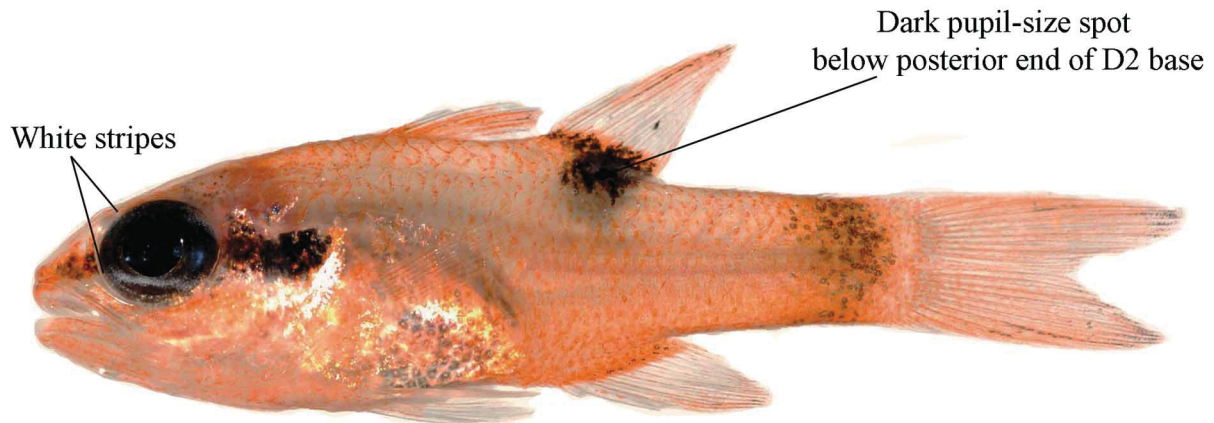
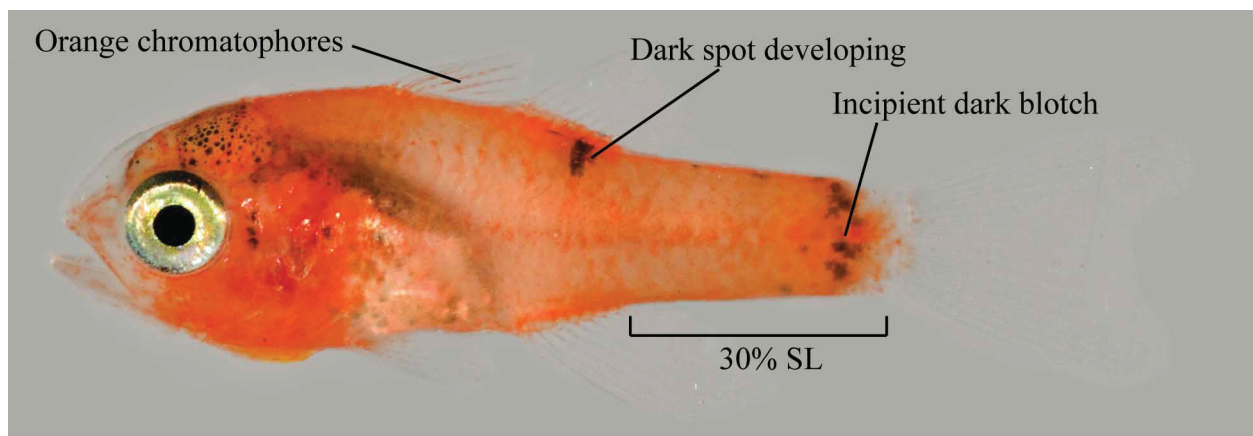


FIGURE 22. *Apogon maculatus*, juvenile, 22.0 mm SL, DNA # BLZ 4551, photograph by J. Mounts and C. Baldwin.

**Comparisons Among Juveniles.** Juvenile *A. maculatus* can be separated from *A. pseudomaculatus* and juveniles of other *Apogon* by characters listed above (see “Comparisons” under *Apogon pseudomaculatus*).

**Larva (Fig. 23).** The single larval specimen, 12.0 mm SL, is largely orange. The fins are mostly clear, but there are orange chromatophores on several rays of the first dorsal fin. There are numerous melanophores on top of the head and behind the eye on the cheek. The dark spot below the posterior portion of the second dorsal-fin base characteristic of juveniles and adults is beginning to develop, and several large, dark melanophores of the incipient caudal-peduncle blotch are present just anterior to the caudal-fin base.

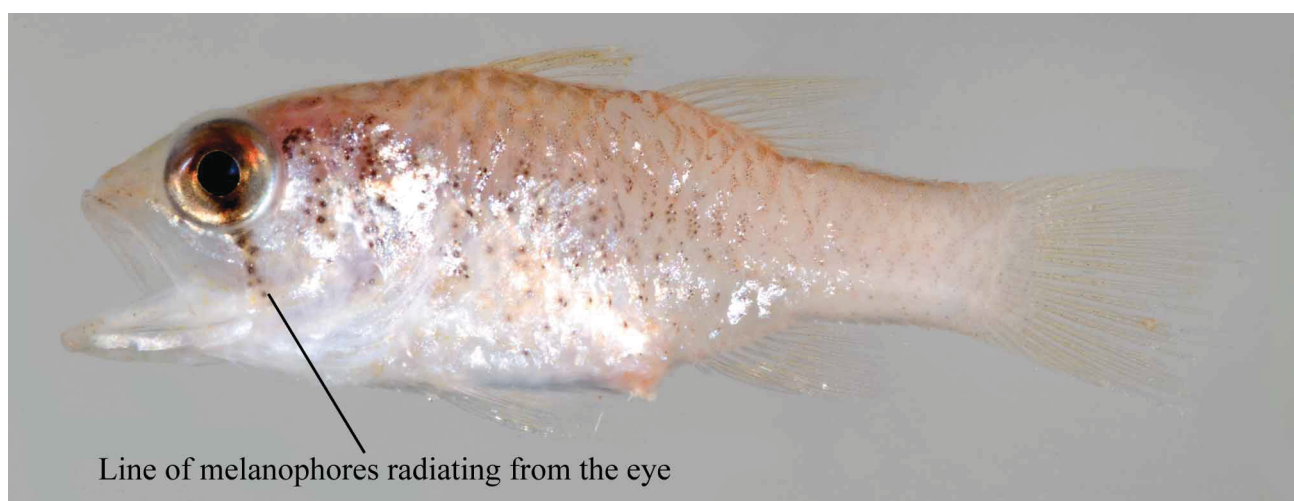


**FIGURE 23.** *Apogon maculatus*, larva 12.0mm SL, DNA # BLZ 7717, photograph by C. Baldwin and L. Weigt;

**Comparisons Among Larvae.** The larval specimen of *A. maculatus* most closely resembles larval *A. aurolineatus* and small *A. phenax* larvae in having a bright orange body color, but it differs from those species and all *Apogon* larvae studied herein in having orange pigment on the first dorsal fin (vs. yellow in *A. aurolineatus*, none in the other species). Additionally, larvae of *A. maculatus* have more melanophores on top of the head than *A. aurolineatus*. Larval *A. maculatus* lacks the conspicuous orange spot above the upper lip of *A. phenax*, the bright yellow pigment on the head of larval *A. planifrons*, and the distinctive pattern of chromatophores on the fins of larval *A. mosavi*. Caudal-peduncle length may be useful in separating preserved larval *A. maculatus* from some other *Apogon* larvae: peduncle length 30% SL in *A. maculatus* vs. 32–40% SL in larval *A. binotatus*, *A. phenax*, *A. planifrons*, *A. townsendi*, and *Apogon* sp. 1. Additionally, the presence of the incipient dark trunk blotches typical of juvenile and adults may indicate precocious development that, in combination with the bright orange body color, could be useful in distinguishing *A. maculatus* larvae from other known *Apogon* species. Among our other *Apogon* larvae, only an 11.0-mm SL specimen of *A. phenax* (Fig. 12c) has the incipient dark bars of juveniles and adults, but the body is considerably paler than in larval *A. maculatus*, especially posteriorly. More larval material is needed.

### *Apogon aurolineatus* (Mowbray)

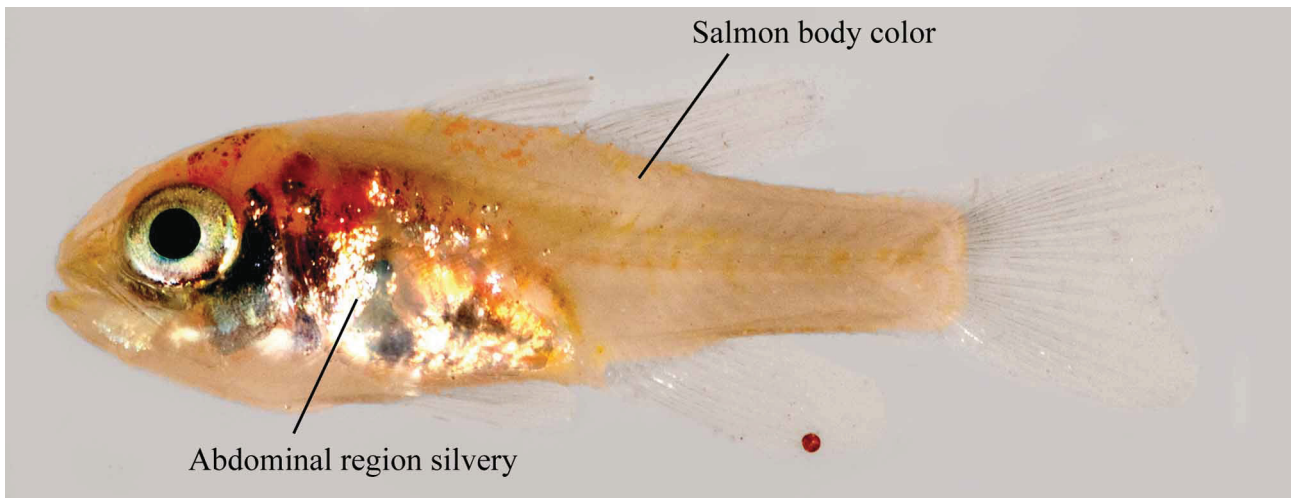
**Identification.** Four adult specimens of *A. aurolineatus* provided the basis for genetic identification of seven larvae and one juvenile (Appendix 1, one adult is shown in Fig. 24). Adult *A. aurolineatus* can be distinguished from other *Apogon* by the combination of eight segmented anal-fin rays, 10–11 gill rakers on the lower limb of the first gill arch, 16–18 circum-caudal-peduncle scales, no dark markings or saddles on the posterior portion of the body, and two to four short dark lines radiating from the eye (Böhlke & Chaplin 1993; Gon 2002).



**FIGURE 24.** *Apogon aurolineatus*, adult, 30.0 mm SL, DNA # BLZ 6176, photograph by J. Mounts and C. Baldwin.



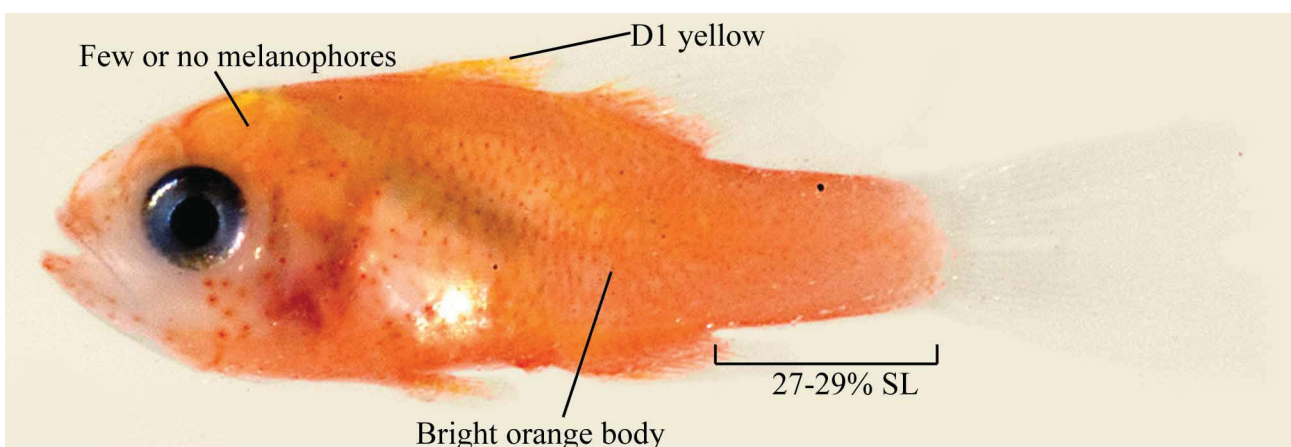
**Juveniles (Fig. 25).** The single juvenile, a reared specimen of 12 mm SL, has a pale salmon body color, and the opercular and abdominal regions are silvery. In preservative, there are no distinctive markings except a few melanophores on top of the head. There are 11 gill rakers on the lower limb of the first gill arch.



**FIGURE 25.** *Apogon aurolineatus*, juvenile, 12.0 mm SL, DNA # BLZ 5497, reared, photograph by L. Weigt.

**Comparisons Among Juveniles.** Of the *Apogon* species for which juveniles are known, *A. aurolineatus* most closely resembles *A. quadrisquamatus* in lacking dark blotches or markings on the body. The reared juvenile of *A. aurolineatus* can be separated from juvenile *A. quadrisquamatus* in having a pale body color (vs. orange in *A. quadrisquamatus*), in lacking yellow on the dorsal and caudal fins, and in lacking an orange spot on the center of the caudal peduncle. Preserved juveniles of the two species are very similar, but eye diameter may be useful in separating the species (diameter of bony orbit approximately 13% SL in the 12.0-mm SL juvenile of *A. aurolineatus* vs. 15% SL in 14.0–16.0-mm SL juveniles of *A. quadrisquamatus*). Preserved juveniles of *A. aurolineatus* also are similar to those of *A. robbyi* and *A. mosavi* in having a pale body, but *A. aurolineatus* lacks the blotch of melanophores on the caudal peduncle present in those species.

**Larvae (Fig. 26).** *Apogon aurolineatus* larvae genetically analyzed in this study are all approximately 8 mm SL. They are bright orange in life and have orange pelvic, anal, and second dorsal fins. The first dorsal fin is orange at the base, but most of the fin is bright yellow. The pectoral and caudal fins are clear. There are some pale areas on the head—below the anterior portion of the eye and above the tip of the snout. The top of the head has yellow pigment in some specimens. There are no dark markings on the body except sometimes a few melanophores on the top of the head. There are barely observable melanophores scattered on the jaws. The caudal-peduncle length ranges from 27 to 29% SL.



**FIGURE 26.** *Apogon aurolineatus*, larva, 8.0 mm SL, DNA # BLZ 5221, photograph by J. Mounts.

**Comparisons Among Larvae.** Fresh specimens of *A. aurolineatus* larvae are easily distinguished from other known *Apogon* larvae by the combination of bright orange body coloration and yellow pigment on the first dorsal fin. Preserved specimens usually have fewer melanophores on top of the head than larvae of other *Apogon* (zero to several vs. many) and a shorter caudal peduncle (27–29 % SL vs. 30–40 % SL in other species).

### *Apogon robinsi* Böhlke and Randall

**Identification.** One adult specimen of *A. robinsi* was collected and analyzed genetically (Appendix 1). Because the photograph of that specimen is not of good quality, we selected a photograph of a specimen not included in the genetic analysis to represent the species (Fig. 27). Adult *A. robinsi* can be distinguished from other *Apogon* species by the combination of eight segmented anal-fin rays, body and lateral-line scales of similar size, body with two distinct dark markings (one bar below and just behind second-dorsal fin and a bar on the posterior part of caudal peduncle—the distance between the two bars larger than the width of the posterior bar), and premaxillary dentition extending outside the mouth laterally on the bone (Böhlke & Chaplin 1993, Gon 2002). No larvae or juveniles analyzed in this study genetically match *A. robinsi*.



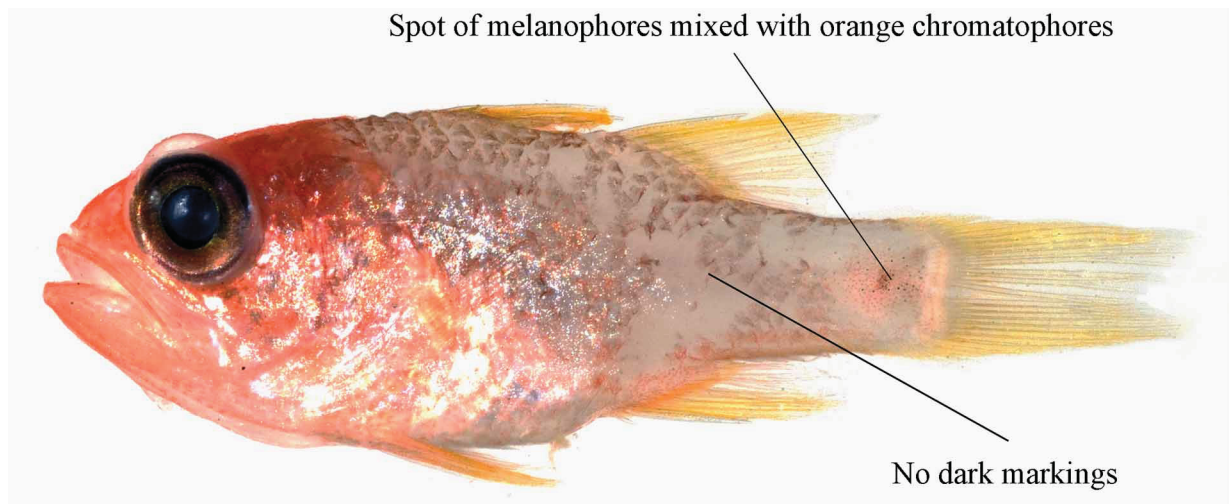
FIGURE 27. *Apogon robinsi*, adult, 35.5 mm SL, USNM 395831, not a DNA voucher, photograph by J. T. Williams.

### *Apogon quadrisquamatus* Longley

#### Lineage A

**Identification.** A single adult specimen from Belize (Fig. 28) constitutes the genetic lineage herein referred to as *Apogon quadrisquamatus* Lineage A. Adult *A. quadrisquamatus* can be distinguished from other *Apogon* by the combination of eight segmented anal-fin rays; lateral-line and body scales of similar size; no dark marking or bar beneath the second dorsal fin; caudal-peduncle spot small, circular, of varying intensity, and usually restricted to middle of caudal peduncle; and 12–14, modally 13, gill rakers on the lower limb of the first arch, (Böhlke & Chaplin 1993, Dale 1977, Gon 2002). The adult specimen in this lineage keys to *Apogon quadrisquamatus* and is distinct from *A. mosavi* and *Apogon* sp. 2 in having a circular blotch of melanophores in the center of the peduncle vs. a rectangular bar (*A. mosavi*) or very diffuse oval (*Apogon* sp. 2) of melanophores. *Apogon robbyi* has a similar circular, basicaudal blotch, but that species is distinctive in having dusky stripes on the trunk. *Apogon quadrisquamatus* Lineage A is further distinguished from *A. mosavi* in having 12 gill rakers on the lower limb of the first arch (possibly a rudiment is forming), vs. 14–15 in *A. mosavi*. The body is mostly orange, and the median fins are yellow. Additional material and further study are needed to determine if this lineage and *A. quadrisquamatus* Lineage B (see next section) are morphologically distinct, and, if so, which one represents *A. quadrisquamatus* Longley 1934. No larvae or juveniles match the single adult specimen of this lineage in our genetic analysis.

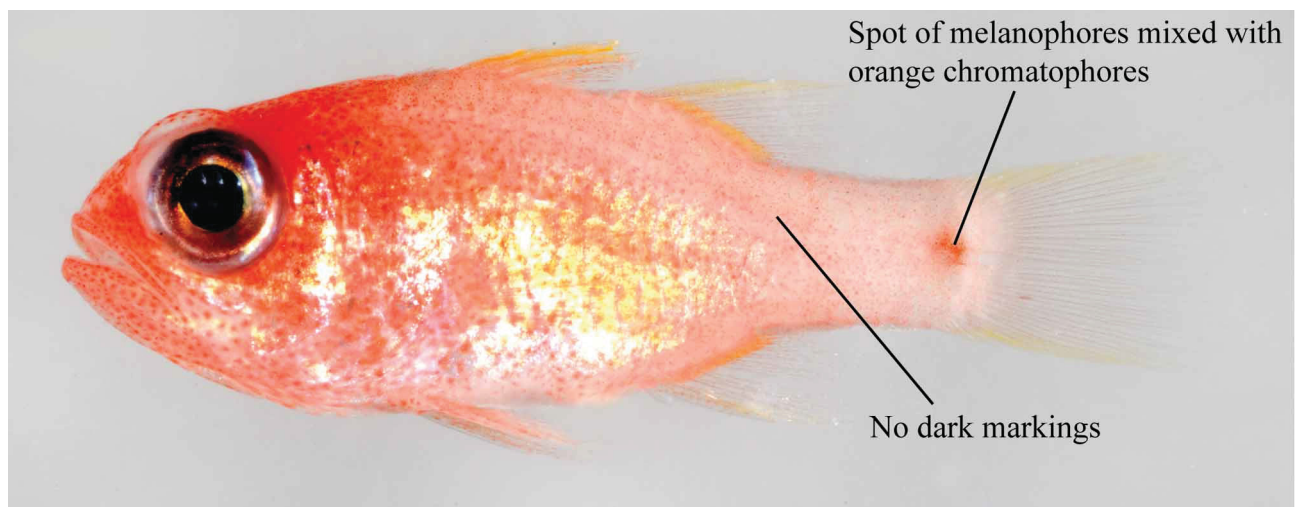




**FIGURE 28.** *Apogon quadrisquamatus* Lineage A, adult, 21.0 mm SL, DNA # BLZ 8291, photograph by C. Baldwin

***Apogon quadrisquamatus* Longley**  
Lineage B

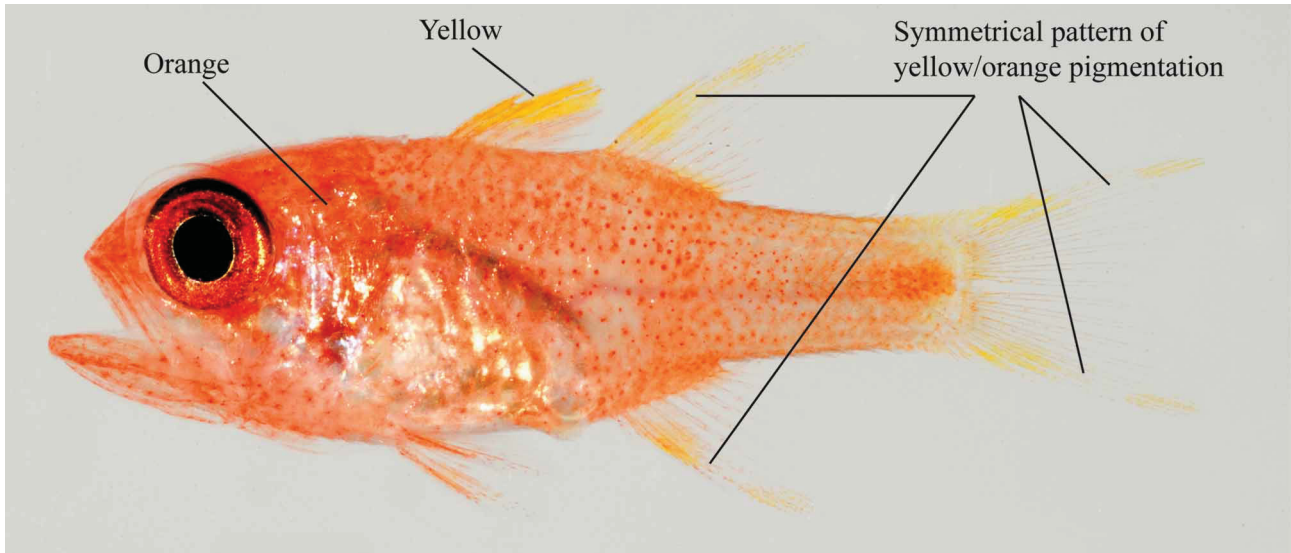
**Identification.** Two additional adult specimens from Belize also were identified as *A. quadrisquamatus* (Appendix 1, one adult is shown in Fig. 29). As noted above, further study of the two *A. quadrisquamatus* lineages is needed. The adult specimens in *A. quadrisquamatus* lineage B provided the basis for genetic identification of three juveniles. All references to *A. quadrisquamatus* juveniles in this paper refer to these three specimens.



**FIGURE 29.** *Apogon quadrisquamatus* Lineage B, adult, 24.0 mm SL, DNA # BLZ 6180, photograph by J. Mounts and C. Baldwin.

**Juveniles (Fig. 30).** Body color in the juveniles (14.0–16.0 mm SL) is mostly orange, and there is a concentration of darker orange pigment in a bar beneath the posterior end of the second dorsal fin and another concentration in a blotch on the caudal peduncle. The entire head, including the snout and jaws, is orange. The dorsal, caudal, anal, and pelvic fins have distinctive blotches of yellow and orange pigment. There is a large yellow/orange blotch covering the entire first dorsal fin. There are two yellow/orange blotches on the anterior portion of the second dorsal fin, one distally and one just above the base of the fin. There are six yellow/orange blotches on the caudal fin: four on the outer caudal-fin rays, two dorsally and two ventrally; and two on the caudal-fin base. There are two yellow/orange blotches on the anterior portion of the anal fin, one distally and one just above the base of the fin. There is one orange blotch on the pelvic fin. There is symmetry in the position of the orange fin blotches such that those on the dorsal fins and dorsal lobe of the caudal fin mirror those on the pelvic fin, anal fin, and ventral lobe of the caudal fin. There are melanophores on top of the head and internally above the swimbladder and gut. There are 12 gill rakers on the lower limb of the first gill arch.

**Comparisons Among Juveniles.** Juveniles of *A. quadrisquamatus* most closely resemble young *A. mosavi*. They can be separated by the color of the fin markings—yellow and orange in *A. quadrisquamatus* vs. entirely orange in *A. mosavi*. Gill rakers on the lower limb of first gill arch are useful in separating preserved juveniles—usually 13 in *A. quadrisquamatus*, 14–15 in *A. mosavi*. *Apogon quadrisquamatus* juveniles also resemble *A. robbyi* juveniles in fin pigmentation, but in the latter this pigment is entirely yellow. *Apogon quadrisquamatus* lacks the orange body stripes characteristic of juvenile *A. robbyi*. Juvenile *A. quadrisquamatus* differs from other *Apogon* species in having the distinctive mirrored pattern of chromatophores on the median fins.



**FIGURE 30.** *Apogon quadrisquamatus* Lineage B, juvenile, 14.0 mm SL, DNA # BLZ 7712, photograph by C. Baldwin and L. Weigt.

### *Apogon* sp. 2

**Identification.** No larvae or juveniles match the five unidentified adult specimens of this lineage in our genetic analysis (Appendix 1, one adult is shown in Fig. 31). The lineage clusters phenetically with *A. quadrisquamatus*, *A. mosavi*, and *A. robbyi* (Fig. 1), and all of those species lack dark markings on the body. There is a concentration of pigment on the central area of the caudal peduncle that is primarily orange and contains few if any melanophores. In preserved specimens this may appear as a diffuse oval blotch of melanophores or no marking at all. In the other species, there is a distinct basicaudal bar (*A. mosavi*) or circular spot (*A. quadrisquamatus* and *A. robbyi*) of melanophores that is retained in preserved specimens. Specimens in this lineage usually have 13 gill rakers on the lower limb of the first arch vs. usually 14 or 15 in *A. mosavi*, but additional material is needed to determine if there are modal differences in any counts. This lineage likely represents an undescribed species.



**FIGURE 31.** *Apogon* sp. 2, adult, 21.1 mm SL, DNA # SAB 0603030, photograph by J. T. Williams.



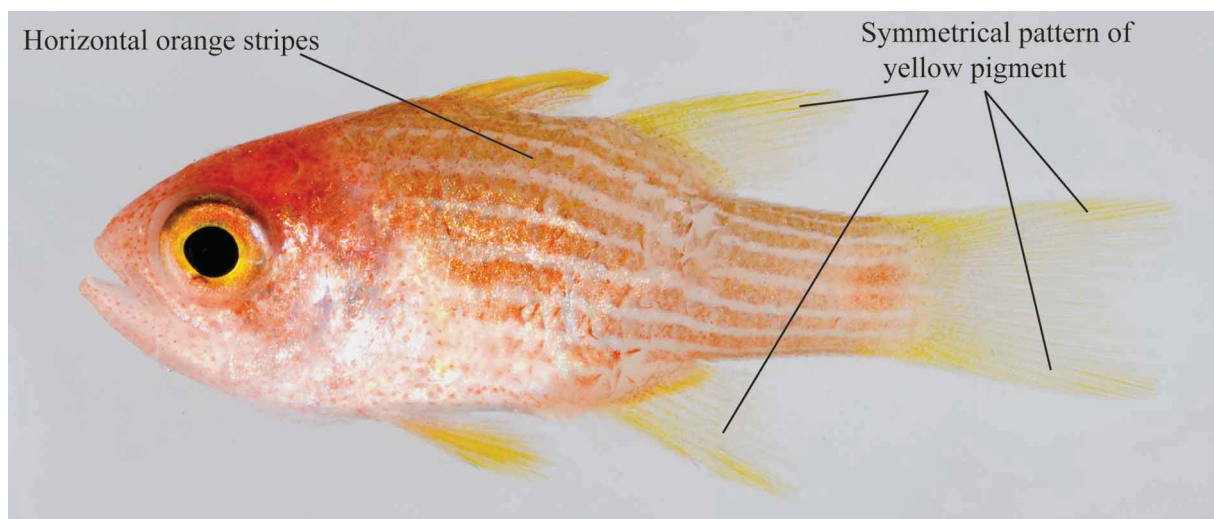
*Apogon robbyi* Gilbert and Tyler

**Identification.** One adult specimen (Fig. 32), identified based on the presence of seven dusky stripes on the body (Gilbert & Tyler, 1997; Gon 2002), served as the basis for genetic identification of two juveniles (Appendix 1).



**FIGURE 32.** *Apogon robbyi*, adult, 27.0 mm SL, DNA # BLZ 6179, photograph by J. Mounts and C. Baldwin.

**Juveniles (Fig. 33).** The juveniles (17.0 and 22.0 mm SL) have the distinctive body stripes of adults, but the stripes are orange and paler than the dusky stripes in adults and not apparent in preserved specimens. There is yellow pigment on the first and second dorsal fins and upper lobe of the caudal fin that roughly mirrors that on the pelvic, anal, and lower lobe of the caudal fin, respectively. There is a round basicaudal spot of melanophores and orange chromatophores that persists in preserved specimens as a well-defined, medially situated, dark blotch. The larger juvenile has 12 gill rakers on the lower limb.

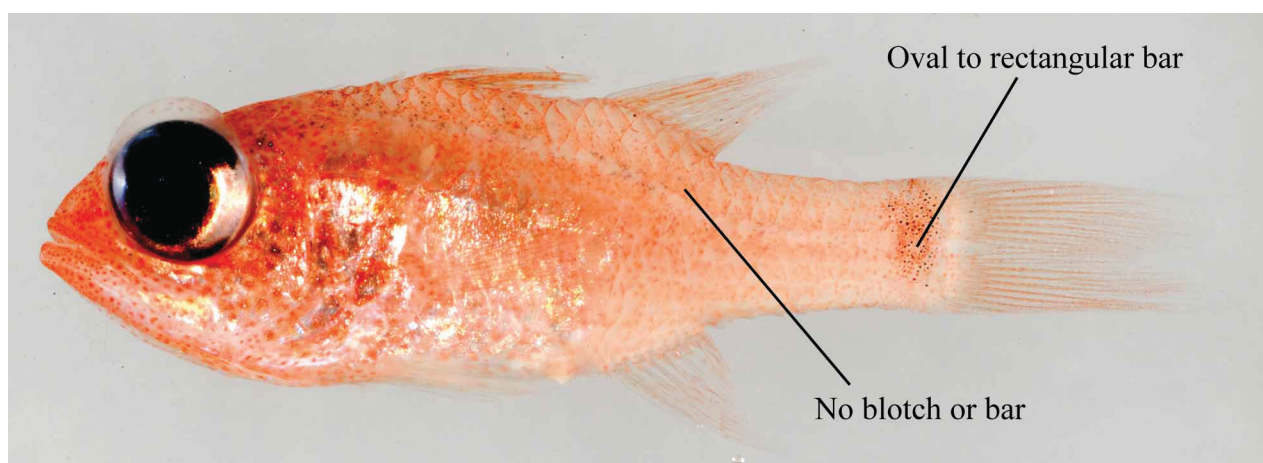


**FIGURE 33.** *Apogon robbyi*, juvenile, 22.0 mm SL, DNA # BLZ 6083, photograph by C. Baldwin and L. Weigt.

**Comparisons Among Juveniles.** Juvenile *A. robbyi* can be separated from other known *Apogon* juveniles by the seven orange-colored stripes on body. It resembles young *A. quadrisquamatus* and *A. mosavi* in having chromatophores on the vertical and pelvic fins, but in *A. robbyi* this pigment is yellow vs. yellow and orange or all orange. As in *A. quadrisquamatus* and *A. mosavi*, the pigment on the dorsal fins and upper caudal lobe appears to mirror that on the pelvic and anal fins and lower lobe of the caudal fin. Modal numbers of gill rakers on the lower limb of the first arch are useful in separating juvenile *A. robbyi* (12–13) from *A. mosavi* (14–15) but not from *A. quadrisquamatus* and *Apogon* sp. 2 (13).

## *Apogon mosavi* Dale

**Identification.** Seventeen adult specimens of *A. mosavi* provided the basis for genetic identification of larvae and juveniles (Appendix 1, one adult is shown in Fig. 34). Adult *A. mosavi* can be distinguished from other *Apogon* species by the combination of eight segmented anal-fin rays, body and lateral-line scales of similar size, no dark marking or bar beneath the second dorsal fin, a rectangular to oval bar present on the caudal peduncle that nearly reaches the dorsal and ventral body margins, and 14–15 (rarely 13 or 16) gill rakers on the lower limb of the first gill arch, (Dale 1977, Gon 2002). Our samples include 20 young specimens of *A. mosavi*, seven of them (14.0–17.0 mm SL) collected in the plankton net and 13 (14.0–20.0 mm SL) with fish anesthetics and dip nets (Appendix 1). As noted in the “Methods” section, we describe those collected in the plankton net as larvae and the others as juveniles. However, until melanophores appear on the caudal peduncle in large juveniles, there are no clear morphological differences between the two stages.



**FIGURE 34.** *Apogon mosavi*, adult, 38.0 mm SL, DNA # BLZ 7131, photograph by J. Mounts.

**Juveniles (Fig. 35).** In the 15.0 to 20.0 mm SL juvenile specimens of *A. mosavi* (Fig. 35a) the body is pale orange and there are no distinctive symmetrical markings on the fins. There is a large blotch of pigment on the caudal peduncle comprising orange chromatophores with sometimes a few melanophores mixed in. There are 14 or 15 gill rakers on the lower limb of the first arch. Fresh specimens of small juveniles (14.0–17.0 mm SL, Fig. 35b) are mostly pale orange, with paler areas on the snout and jaws, beneath the anterior portion of the second dorsal fin, and on the anterior portion of the caudal peduncle. The dorsal, caudal, anal, and pelvic fins have distinctive blotches of orange pigment. There is a large orange blotch covering the entire first dorsal fin except the bases of the rays. Three orange blotches are present on the second dorsal fin—two on the anterior portion of the fin and the third on the posterior base of the fin. There are six orange blotches on the caudal fin: four on the outer caudal-fin rays, two dorsally and two ventrally, and two on the caudal-fin base. Three orange are present blotches on the anal fin—two on the anterior portion of the fin and the third on the posterior base of the fin. There is one orange blotch on the pelvic fin. There is symmetry in the position of the orange fin blotches such that those on the dorsal fins and dorsal lobe of the caudal fin mirror those on the pelvic fin, anal fin, and ventral lobe of the caudal fin. There are melanophores on top of the head and internally above the gut.

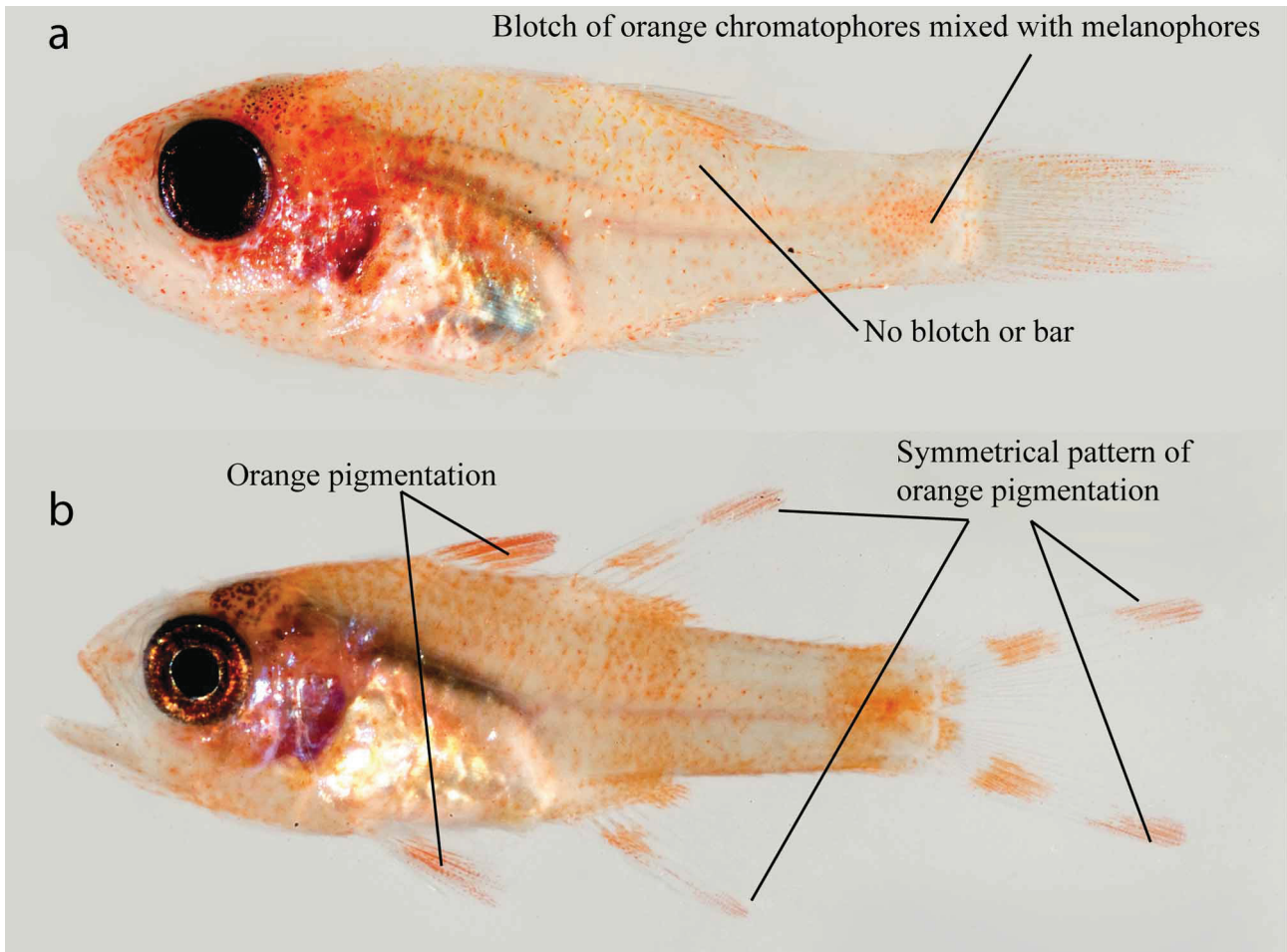
**Comparisons Among Juveniles.** Young juveniles of *A. mosavi* most closely resemble those of *A. quadrisquamatus* and *A. robbyi* in having distinctive patterns of chromatophores on the fins. See “Comparisons” under *A. quadrisquamatus* and *A. robbyi* juveniles for characters that distinguish them.

**Larvae (Fig. 36).** The seven larvae (14.0–17.0 mm SL) collected in the plankton have the same patterns of chromatophores and melanophores as those described above for small juveniles. The caudal-peduncle length ranges from 31–34% SL.

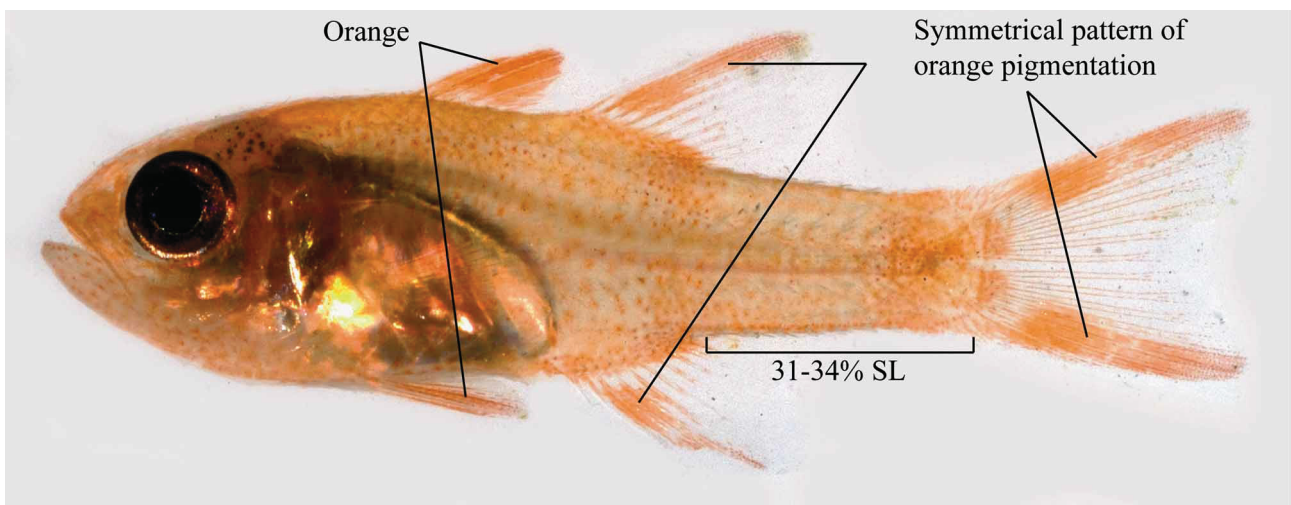
**Comparisons Among Larvae.** Adult *A. mosavi* are most similar to *A. quadrisquamatus* and *Apogon* sp. 2. Larvae of *A. quadrisquamatus* are unknown, but juvenile *A. quadrisquamatus* and larval and juvenile *A. mosavi* have a similar pattern of fin pigment, suggesting that the larvae of *A. quadrisquamatus* may as well. Assuming the color of fin pigment of larval *A. quadrisquamatus* is the same as it is in juveniles, as is the case in *A. mosavi*, lar-



val *A. mosavi* will differ from larval *A. quadrisquamatus* in having orange pigment on the fins (vs. orange and yellow). Likewise, larvae of *A. robbyi* are unknown, but they should differ from *A. mosavi* larvae in having yellow vs. orange fin pigment. The pattern of fin pigment in larval *A. mosavi* is sufficient to separate that species from larvae of other known *Apogon*. We know of no morphological features of preserved *A. mosavi* larvae that separate them from other *Apogon* larvae except the numerous melanophores on top of the head and long caudal peduncle (31–34% SL) will distinguish them from larval *A. aurolineatus* (few or no melanophores on top of the head and caudal-peduncle length 27–29% SL).



**FIGURE 35.** *Apogon mosavi* a) juvenile, 15.5 mm SL, DNA # BLZ 7713, fresh specimen, photograph by C. Baldwin and L. Weigt; b) juvenile, 15.0 mm SL, DNA # BLZ 7122, photograph by J. Mounts.



**FIGURE 36.** *Apogon mosavi*, larva, 15.0 mm SL, DNA # BLZ 5454, photograph by L. Weigt.



## Discussion

Larvae of *A. aurolineatus*, *A. binotatus*, *A. maculatus*, *A. mosavi*, *A. phenax*, *A. planifrons*, *A. townsendi*, and an unidentified species, and juveniles of *A. aurolineatus*, *A. binotatus*, *A. lachneri*, *A. maculatus*, *A. mosavi*, *A. phenax*, *A. pillionatus*, *A. pseudomaculatus*, *A. robbyi*, *A. townsendi*, and *A. quadrisquamatus* are described herein. Preserved *Apogon* larvae are difficult to identify because there are few diagnostic characters that separate the species. Only two characters were discovered in this study that are useful in separating preserved *Apogon* larvae, and both separate *A. aurolineatus* from other known *Apogon* larvae: in *A. aurolineatus* there are few or no melanophores on the top of the head (vs. many melanophores in all other species), and the caudal peduncle is short (27–29% SL vs 31–40% SL in most other *Apogon* larvae). The longest caudal peduncle is found in *A. planifrons* (35–40% SL), but *A. phenax*, *A. binotatus*, and *A. townsendi* also have a long peduncle (32–37% SL). The single larval specimen of *A. maculatus* has a relatively short caudal peduncle (30% SL), and that feature may be of value in distinguishing *A. maculatus* from some species. More material is needed. Chromatophore patterns are the easiest, and frequently only, way to separate and identify larval stages of most *Apogon* species morphologically, but not all species can be distinguished this way based on material examined in this study. For example, we found no consistent features to distinguish *Apogon binotatus*, *A. townsendi*, and *Apogon* sp. 1.

Preserved juveniles of *Apogon* species are easier to identify because many exhibit the diagnostic pigment patterns of adults. However, when adult pigmentation is not fully developed, some species can be confused. For example, the posterior dark bar in *A. binotatus*, which is as narrow as the anterior bar in adults, is broader than the anterior bar in juveniles. Accordingly, juvenile *A. binotatus* may be confused with juvenile *A. phenax*. Chromatophore patterns in fresh specimens, in combination with patterns of melanophores, often provide the best means of separating juvenile *Apogon*. Because juveniles have the full adult complement of gill rakers, the number of lower-limb rakers can be used to identify some *Apogon* juveniles.

*Apogon* larvae (and young juveniles) can be sorted into at least four morphological groups: (1) *A. aurolineatus*, (2) *A. maculatus*, (3) *A. quadrisquamatus* group (*A. quadrisquamatus*, *A. robbyi*, *A. mosavi*), and (4) *A. planifrons* group (*A. binotatus*, *A. pillionatus*, *Apogon* sp. 1, *A. planifrons*, *A. phenax*, and *A. townsendi*). *Apogon aurolineatus* larvae have bright orange second dorsal, anal, and pelvic fins, a bright yellow first dorsal fin, few if any melanophores on top of the head, and a short caudal peduncle (27 to 29% SL).

The single specimen of larval *A. maculatus* has conspicuous orange pigment on the first dorsal fin, a relatively short caudal peduncle (30% SL), and possibly precocious development of the trunk blotches diagnostic of adults. Larvae of *A. pseudomaculatus* are unknown, but adults are similar to *A. maculatus* in having a spot or blotch of pigment beneath the posterior base of the second dorsal fin (vs. no pigment or a dark bar), and presence of the diagnostic features of larval *A. maculatus* in young *A. pseudomaculatus* would support its placement in the *A. maculatus* morphological group.

Young stages of the *A. quadrisquamatus* group are distinctive in exhibiting symmetry in the position of orange or orange and yellow blotches of pigment on the fins, such that those on the first and second dorsal fins and dorsal lobe of the caudal fin mirror those on the pelvic fin, anal fin, and ventral lobe of the caudal fin, respectively. Obviously different from *A. mosavi* and *A. quadrisquamatus* because of the striped pattern on the body, young *A. robbyi* also have symmetrical pattern of fin pigment (yellow vs. orange or yellow/orange). In young of all three species there is a blotch of pale orange chromatophores, sometimes with a few melanophores mixed in, on the central portion of the caudal peduncle. There are numerous melanophores on top of the head, and the caudal peduncle is relatively long (>30% SL). Young stages of *Apogon* sp. 2 are unknown, but adults are similar morphologically to members of the *A. quadrisquamatus* group, and presence of the diagnostic features of larvae and juveniles of that group in young *Apogon* sp. 2 would corroborate its inclusion in the *A. quadrisquamatus* morphological group. In the *A. planifrons* group, the vertical fins are never bright orange/yellow as in larval *A. aurolineatus*, and they lack distinctive orange color on the first dorsal fin characteristic of *A. maculatus*. Chromatophores may be present on the fins in juveniles, but they are not in symmetrical dorsal/ventral patterns as in the *A. quadrisquamatus* group. The posterior portion of the head is usually brightly colored, and it may be mostly orange or mostly yellow. There are often pale areas on the snout and on posterior portions of the trunk. There are typically numerous melanophores on top of the head, and the caudal peduncle is relatively long (> 30% SL).

As noted, juveniles of *A. lachneri* resemble juvenile *A. maculatus* and *A. pseudomaculatus* in having a blotch of pigment beneath the posterior end of the second dorsal fin (vs. a bar or no pigment in the other species), but they

are unique in having conspicuous dark pigment on the dorsal and anal fins. *Apogon lachneri* may represent a fifth morphological group of western Central Atlantic *Apogon* larvae. The absence of any early life-history stages of *A. affinis* and *A. robinsi* in our material precludes comments on their affinities with any of the morphological groups described above.

In Thacker and Roje's (2009) molecular phylogeny of cardinalfishes, *Apogon quadrisquamatus*, *A. aurolineatus*, and *A. maculatus* constitute genetic lineages that, together with *Astrapogon puncticulatus* and *Phaeoptyx conklini*, form a monophyletic clade. However, the three *Apogon* species do not constitute a monophyletic group; rather, *Apogon quadrisquamatus* is the sister group of *P. conklini*, and *Apogon maculatus* is the sister group of *Astrapogon puncticulatus*. Although additional phylogenetic analyses that incorporate more taxa are needed, the molecular phylogenetic data suggest that western Atlantic *Apogon* may not be monophyletic and that there may be several distinct genetic lineages within the genus. The morphological groups of larvae identified herein could also indicate the existence of multiple clades within western Atlantic *Apogon*, but a resolved phylogeny is needed to determine if any of the larval groups are monophyletic. Ideally further study will include the three Caribbean *Apogon* species not represented in our genetic material—*Apogon evermanni*, *A. leptocaulus*, and *A. gouldi*, as well as the eastern Atlantic *A. imberbis*, the central Atlantic (Ascension/St. Helena) *A. axillaris*, and the Brazilian *S. americanus*.

## Conclusions

Larvae and juveniles of closely related reef-fish species are often difficult to identify because they lack the full complement of adult diagnostic features. DNA barcoding proved effective in matching young stages and adults of western Atlantic *Apogon* species, and subsequent study of preserved larval and juvenile voucher specimens or color photographs of them taken prior to preservation illuminated diagnostic morphological characters of the young stages. Characters that are most useful in separating larvae of *Apogon* species are patterns of chromatophores. Patterns of melanophores and morphometrics unfortunately are of limited value, rendering preserved *Apogon* larvae in existing museum collections difficult or impossible to identify. Characters that are most useful in separating juveniles are patterns of chromatophores, melanophores, and numbers of lower-limb gill rakers on the first arch.

The ability to identify young stages of coral-reef fishes by genetically matching them to adults using a relatively short, typically easily obtained DNA sequence should greatly increase the number of such identifications made in the future. The identification of at least four morphological groups of early life history stages of western Atlantic *Apogon* herein may corroborate previous phylogenetic work that suggests that multiple clades exist within the genus. As with many marine teleost groups (e.g., Ahlstrom *et al.* 1984), apogonid larvae may contribute new information of value in reconstructing phylogenetic relationships.

## Acknowledgements

We thank A. Driskell and A. Ormos for laboratory and logistical assistance; M. Carpenter, A. Driskell, C. DeCourley, Z. Foltz, J. Lang, L. Lang, D. Miller, J. Mounts, and R. Murphy for assistance in the field; P. Díaz, M. Lavín, L. Sánchez and A. Kobelkowsky for their guidance of the first author; J. T. Williams, J. Van Tassell, and D. R. Robertson for providing images; D. R. Robertson, D. Griswold, and C. Castillo for help with image editing; the Smithsonian Marine Science Network and the National Museum of Natural History Small-Grants Program for financial support to the first author; the Consejo Nacional de Ciencia y Tecnología (CONACyT) for financial support for the second author to conduct research at the Smithsonian's National Museum of Natural History; PAPIIT IN 207410 for support granted to E. Escobar; and the Dirección General de Posgrados (DGP, UNAM) for financial support within the International Exchange Student Program. Research in Florida was conducted pursuant to SAL # 07SR-1024B to the first author. A. Gazit, K. Wilson, and M. Kunen facilitated collecting in Curaçao through the CARM-ABI laboratory. Fieldwork in the Bahamas was conducted under the auspices of the Perry Institute of Marine Science, with logistical assistance from B. Gadd, E. Lamarre, and D. O'Donnell. This is Caribbean Coral Reef Ecosystems Program (CCRE) contribution number XXX and Smithsonian Marine Station at Ft. Pierce, FL (SMSFP) contribution number XXX.

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**Appendix 1.** *Apogon* material analyzed for mitochondrial cytochrome oxidase-*c* subunit I. BAH = Bahamas; BLZ = Belize; CUR = Curaçao; FCC, FWRI, and SMS = Florida; SAB = Saba Bank, Netherland Antilles. The DNA number is listed as “field number” in the Barcode of Life Data Systems database (<http://www.boldsystems.org>).

DNA NUMBER	SPECIES	Life cycle stage	SL (mm)	SPECIMEN VOUCHER	PHOTO VOUCHER
BAH 8072	<i>Apogon binotatus</i>	A	36.0	USNM 401815	Yes
BAH 8073	<i>Apogon binotatus</i>	A	36.0	USNM 401816	Yes
BAH 8074	<i>Apogon binotatus</i>	A	32.0	USNM 401817	Yes
BLZ 4446	<i>Apogon binotatus</i>	L	9.0	No	No
BLZ 4574	<i>Apogon binotatus</i>	J	14.0	No	Yes
BLZ 5426	<i>Apogon binotatus</i>	L	9.5	No	Yes
BLZ 6203	<i>Apogon binotatus</i>	L	10.0	No	No
BLZ 6325	<i>Apogon binotatus</i>	A	46.0	USNM 401818	Yes
BLZ 6326	<i>Apogon binotatus</i>	A	42.0	USNM 401819	Yes
BLZ 6327	<i>Apogon binotatus</i>	A	33.0	USNM 401820	Yes
BLZ 6330	<i>Apogon binotatus</i>	L	9.0	No	Yes
BLZ 6331	<i>Apogon binotatus</i>	L	9.0	No	Yes
BLZ 6332	<i>Apogon binotatus</i>	L	9.0	No	Yes
BLZ 6360	<i>Apogon binotatus</i>	J	13.5	No	Yes
BLZ 6373	<i>Apogon binotatus</i>	A	26.0	USNM 401951	Yes
BLZ 6398	<i>Apogon binotatus</i>	L	8.5	No	Yes
BLZ 7009	<i>Apogon binotatus</i>	L	11.0	USNM 401821	Yes
BLZ 7010	<i>Apogon binotatus</i>	L	9.5	USNM 401822	Yes
BLZ 7018	<i>Apogon binotatus</i>	L	10.0	USNM 401823	Yes
BLZ 7075	<i>Apogon binotatus</i>	L	8.8	No	Yes
BLZ 7123	<i>Apogon binotatus</i>	J	14.0	USNM 401824	Yes
BLZ 7197	<i>Apogon binotatus</i>	L	10.5	No	Yes
BLZ 8319	<i>Apogon binotatus</i>	J	18.0	USNM 401825	Yes
BLZ 6228	<i>Apogon binotatus</i>	A	39.0	USNM 401826	Yes
CUR 8080	<i>Apogon binotatus</i>	A	35.0	USNM 401827	Yes
CUR 8081	<i>Apogon binotatus</i>	A	40.0	USNM 401828	Yes
CUR 8328	<i>Apogon binotatus</i>	A	34.0	USNM 401829	Yes
SMS 7686	<i>Apogon binotatus</i>	J	14.0	USNM 401954	Yes
BLZ 5056	<i>Apogon pillionatus</i>	J	16.0	No	Yes
BLZ 5057	<i>Apogon pillionatus</i>	J	17.0	USNM 401830	Yes
BLZ 8112	<i>Apogon pillionatus</i>	J	16.0	USNM 401831	Yes
BLZ 8144	<i>Apogon pillionatus</i>	J	15.0	USNM 401832	Yes
CUR 8098	<i>Apogon pillionatus</i>	J	17.0	USNM 401833	Yes
BLZ 5260	<i>Apogon</i> sp. 1	L	10.0	No	Yes
BLZ 5270	<i>Apogon planifrons</i>	A	37.0	USNM 401834	Yes
BLZ 5389	<i>Apogon planifrons</i>	L	10.0	USNM 401835	Yes
BLZ 6042	<i>Apogon planifrons</i>	L	9.0	USNM 401836	Yes
BLZ 6427	<i>Apogon planifrons</i>	L	10.0	No	Yes
BLZ 7045	<i>Apogon planifrons</i>	L	9.5	No	Yes
BLZ 7126	<i>Apogon planifrons</i>	L	10.0	No	Yes
BAH 8075	<i>Apogon phenax</i>	A	45.0	USNM 401837	Yes
BAH 8076	<i>Apogon phenax</i>	A	27.0	USNM 401838	Yes
BLZ 5055	<i>Apogon phenax</i>	A	28.0	USNM 401839	Yes
BLZ 5173	<i>Apogon phenax</i>	A	28.0	USNM 401955	Yes
BLZ 5268	<i>Apogon phenax</i>	A	32.0	USNM 401840	Yes
BLZ 6147	<i>Apogon phenax</i>	J	22.0	USNM 401841	Yes

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**Appendix 1 (continued)**

DNA NUMBER	SPECIES	Life cycle stage	SL (mm)	SPECIMEN VOUCHER	PHOTO VOUCHER
BLZ 6148	<i>Apogon phenax</i>	J	20.0	USNM 401842	Yes
BLZ 6286	<i>Apogon phenax</i>	A	35.0	USNM 401843	Yes
BLZ 6335	<i>Apogon phenax</i>	L	9.5	No	Yes
BLZ 6355	<i>Apogon phenax</i>	A	35.0	USNM 401844	Yes
BLZ 6359	<i>Apogon phenax</i>	L	11.0	No	Yes
BLZ 6361	<i>Apogon phenax</i>	L	10.0	No	Yes
BLZ 7051	<i>Apogon phenax</i>	L	11.0	USNM 401846	Yes
BLZ 7056	<i>Apogon phenax</i>	L	9.5	No	Yes
BLZ 7076	<i>Apogon phenax</i>	L	9.5	No	Yes
BLZ 7153	<i>Apogon phenax</i>	J	12.5	No	Yes
BLZ 7167	<i>Apogon phenax</i>	L	11.0	No	Yes
BLZ 7234	<i>Apogon phenax</i>	J	18.0	USNM 401847	Yes
BLZ 7736	<i>Apogon phenax</i>	J	19.0	USNM 401848	Yes
BLZ 7737	<i>Apogon phenax</i>	A	36.0	USNM 401849	Yes
BLZ 8051	<i>Apogon phenax</i>	A	27.0	USNM 401850	Yes
BLZ 8079	<i>Apogon phenax</i>	A	29.0	USNM 401851	Yes
BLZ 8111	<i>Apogon phenax</i>	J	19.0	USNM 401852	Yes
BLZ 8166	<i>Apogon phenax</i>	J	16.0	USNM 401853	Yes
BLZ 8229	<i>Apogon phenax</i>	J	16.0	USNM 401854	Yes
CUR 8067	<i>Apogon phenax</i>	A	48.0	USNM 401855	Yes
CUR 8088	<i>Apogon phenax</i>	A	29.0	USNM 401856	Yes
CUR 8089	<i>Apogon phenax</i>	A	34.0	USNM 401857	Yes
CUR 8090	<i>Apogon phenax</i>	A	30.0	USNM 401858	Yes
CUR 8322	<i>Apogon phenax</i>	A	50.0	USNM 401859	Yes
CUR 8323	<i>Apogon phenax</i>	A	37.5	USNM 401860	Yes
BLZ 5059	<i>Apogon lachneri</i>	J	19.0	USNM 401861	Yes
BLZ 5060	<i>Apogon lachneri</i>	J	18.0	USNM 401862	Yes
BLZ 5118	<i>Apogon lachneri</i>	A	36.0	USNM 401863	Yes
BLZ 5119	<i>Apogon lachneri</i>	J	18.0	USNM 401864	Yes
BLZ 5264	<i>Apogon lachneri</i>	J	22.0	USNM 401865	Yes
BLZ 5265	<i>Apogon lachneri</i>	J	21.0	USNM 401866	Yes
BLZ 5266	<i>Apogon lachneri</i>	J	21.0	USNM 401867	Yes
BLZ 7829	<i>Apogon lachneri</i>	J	22.0	USNM 401868	Yes
CUR 8075	<i>Apogon lachneri</i>	A	32.0	USNM 401869	Yes
CUR 8076	<i>Apogon lachneri</i>	A	39.0	USNM 401870	Yes
CUR 8324	<i>Apogon lachneri</i>	A	24.0	USNM 401871	Yes
BAH 8065	<i>Apogon townsendi</i>	A	30.0	USNM 0401876	Yes
BLZ 4542	<i>Apogon townsendi</i>	J	13.0	No	Yes
BLZ5002	<i>Apogon townsendi</i>	J	16.0	USNM 401877	Yes
BLZ 5101	<i>Apogon townsendi</i>	J	19.0	USNM 401878	Yes
BLZ 5148	<i>Apogon townsendi</i>	J	21.0	USNM 401879	Yes
BLZ 5149	<i>Apogon townsendi</i>	J	19.0	USNM 401880	Yes
BLZ 5150	<i>Apogon townsendi</i>	J	14.0	USNM 401881	Yes
BLZ 5502	<i>Apogon townsendi</i>	J	14.0	USNM 401882	Yes
BLZ 6127	<i>Apogon townsendi</i>	J	19.0	USNM 401883	Yes
BLZ 6128	<i>Apogon townsendi</i>	J	16.0	USNM 401884	Yes
BLZ 6295	<i>Apogon townsendi</i>	A	31.0	USNM 401963	Yes
BLZ 6296	<i>Apogon townsendi</i>	A	24.0	USNM 401964	Yes
BLZ 6329	<i>Apogon townsendi</i>	L	11.0	No	Yes
BLZ 6333	<i>Apogon townsendi</i>	L	11.0	No	Yes

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**Appendix 1** (continued)

DNA NUMBER	SPECIES	Life cycle stage	SL (mm)	SPECIMEN VOUCHER	PHOTO VOUCHER
BLZ 6334	<i>Apogon townsendi</i>	J	12.0	No	Yes
BLZ 6354	<i>Apogon townsendi</i>	A	37.0	USNM 401885	Yes
BLZ 7832	<i>Apogon townsendi</i>	A	34.0	USNM 401886	Yes
BLZ 7833	<i>Apogon townsendi</i>	A	32.0	USNM 401887	Yes
BLZ 7834	<i>Apogon townsendi</i>	A	31.0	USNM 401888	Yes
CUR 8068	<i>Apogon townsendi</i>	A	35.0	USNM 401889	Yes
CUR 8069	<i>Apogon townsendi</i>	A	34.0	USNM 401890	Yes
CUR 8070	<i>Apogon townsendi</i>	A	32.0	USNM 401891	Yes
CUR 8071	<i>Apogon townsendi</i>	A	33.0	USNM 401892	Yes
CUR 8072	<i>Apogon townsendi</i>	J	14.5	USNM 401893	Yes
CUR 8073	<i>Apogon townsendi</i>	J	17.0	USNM 401894	Yes
CUR 8074	<i>Apogon townsendi</i>	J	15.5	USNM 401895	Yes
SAB 0611075	<i>Apogon townsendi</i>	A	39.1	USNM 397414	No
BLZ 4550	<i>Apogon pseudomaculatus</i>	J	23.0	USNM 401956	Yes
CUR 8079	<i>Apogon pseudomaculatus</i>	J	19.0	USNM 401901	Yes
FWRI 20646	<i>Apogon pseudomaculatus</i>	A	55.0	FSBC 020646	Yes
FWRI 20664a	<i>Apogon affinis</i>	A	57.0	FSBC 020664a	Yes
FWRI 20664b	<i>Apogon affinis</i>	A	52.0	FSBC 020664b	Yes
BAH 8241	<i>Apogon maculatus</i>	A	27.0	USNM 401896	Yes
BLZ 4170	<i>Apogon maculatus</i>	A	38.0	No	No
BLZ 4293	<i>Apogon maculatus</i>	A	38.0	No	No
BLZ 4321	<i>Apogon maculatus</i>	A	32.0	No	No
BLZ 4551	<i>Apogon maculatus</i>	J	22.0	No	Yes
BLZ 5023	<i>Apogon maculatus</i>	A	28.0	USNM 401897	Yes
BLZ 5267	<i>Apogon maculatus</i>	A	29.0	USNM 401898	Yes
BLZ 7717	<i>Apogon maculatus</i>	L	12.0	No	Yes
CUR 8077	<i>Apogon maculatus</i>	A	39.0	USNM 401899	Yes
CUR 8078	<i>Apogon maculatus</i>	J	22.0	USNM 401900	Yes
FCC 8117	<i>Apogon maculatus</i>	A	62.0	No	No
SAB 0601005	<i>Apogon maculatus</i>	A	55.0	USNM 387764	No
SAB 0612078	<i>Apogon maculatus</i>	A	21.1	USNM 397417	No
SMS 7211	<i>Apogon maculatus</i>	J	16.0	USNM 401962	Yes
SMS 7370	<i>Apogon maculatus</i>	A	85.0	USNM 401957	Yes
SMS 7371	<i>Apogon maculatus</i>	A	70.0	USNM 401958	Yes
SMS 7372	<i>Apogon maculatus</i>	A	68.0	USNM 401959	Yes
SMS 7373	<i>Apogon maculatus</i>	A	75.0	USNM 401960	Yes
SMS 7374	<i>Apogon maculatus</i>	A	60.0	USNM 401961	Yes
BLZ 4033	<i>Apogon aurolineatus</i>	L	-	No	No
BLZ 4054	<i>Apogon aurolineatus</i>	L	-	No	No
BLZ 4055	<i>Apogon aurolineatus</i>	L	-	No	No
BLZ 4092	<i>Apogon aurolineatus</i>	L	-	No	No
BLZ 5014	<i>Apogon aurolineatus</i>	L	-	No	Yes
BLZ 5221	<i>Apogon aurolineatus</i>	L	8.0	No	Yes
BLZ 5222	<i>Apogon aurolineatus</i>	L	8.0	No	Yes
BLZ 5497	<i>Apogon aurolineatus</i>	J	12.0	USNM 401902	Yes

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**Appendix 1** (continued)

DNA NUMBER	SPECIES	Life cycle stage	SL (mm)	SPECIMEN VOUCHER	PHOTO VOUCHER
BLZ 6176	<i>Apogon aurolineatus</i>	A	30.0	USNM 401903	Yes
BLZ 6177	<i>Apogon aurolineatus</i>	A	30.0	USNM 401904	Yes
BLZ 7325	<i>Apogon aurolineatus</i>	A	28.0	USNM 401905	Yes
BLZ 7738	<i>Apogon aurolineatus</i>	A	26.0	USNM 401906	Yes
BAH 8142	<i>Apogon robinsi</i>	A	63.0	USNM 401907	Yes
BLZ 8291	<i>Apogon quadrisquamatus</i> Lineage A	A	21.0	USNM 401908	Yes
BLZ 6085	<i>Apogon quadrisquamatus</i> Lineage B	J	14.0	USNM 401909	Yes
BLZ 6180	<i>Apogon quadrisquamatus</i> Lineage B	A	24.0	USNM 401910	Yes
BLZ 6181	<i>Apogon quadrisquamatus</i> Lineage B	A	29.0	USNM 401911	Yes
BLZ 7196	<i>Apogon quadrisquamatus</i> Lineage B	J	16.0	No	Yes
BLZ 7712	<i>Apogon quadrisquamatus</i> Lineage B	J	14.0	USNM 401912	Yes
BAH 8080	<i>Apogon</i> sp. 2	A	43.0	USNM 401913	Yes
BAH 8081	<i>Apogon</i> sp. 2	A	35.0	USNM 401914	Yes
BAH 8082	<i>Apogon</i> sp. 2	A	34.0	USNM 401915	Yes
BLZ 6112	<i>Apogon</i> sp. 2	A	39.0	USNM 401916	Yes
SAB 0603030	<i>Apogon</i> sp. 2	A	21.1	USNM 387832	Yes
BLZ 6083	<i>Apogon robbyi</i>	J	22.0	USNM 401917	Yes
BLZ 6084	<i>Apogon robbyi</i>	J	17.0	USNM 401918	Yes
BLZ 6179	<i>Apogon robbyi</i>	A	27.0	USNM 401919	Yes
BLZ 5454	<i>Apogon mosavi</i>	L	15.0	USNM 401920	Yes
BLZ 6182	<i>Apogon mosavi</i>	J	20.0	USNM 401845	Yes
BLZ 6275	<i>Apogon mosavi</i>	J	20.0	USNM 401921	Yes
BLZ 6403	<i>Apogon mosavi</i>	J	15.0	USNM 401922	Yes
BLZ 6413	<i>Apogon mosavi</i>	L	17.0	No	Yes
BLZ 7113	<i>Apogon mosavi</i>	A	30.0	USNM 401923	Yes
BLZ 7114	<i>Apogon mosavi</i>	A	26.0	USNM 401924	Yes
BLZ 7115	<i>Apogon mosavi</i>	A	27.0	USNM 401925	Yes
BLZ 7116	<i>Apogon mosavi</i>	A	24.0	USNM 401926	Yes
BLZ 7117	<i>Apogon mosavi</i>	A	23.0	USNM 401927	Yes
BLZ 7118	<i>Apogon mosavi</i>	A	24.0	USNM 401928	Yes
BLZ 7119	<i>Apogon mosavi</i>	A	23.0	USNM 401929	Yes
BLZ 7120	<i>Apogon mosavi</i>	J	18.0	USNM 401930	Yes
BLZ 7121	<i>Apogon mosavi</i>	J	17.0	USNM 401931	Yes
BLZ 7122	<i>Apogon mosavi</i>	J	15.0	No	Yes
BLZ 7131	<i>Apogon mosavi</i>	A	38.0	USNM 401932	Yes

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**Appendix 1** (continued)

DNA NUMBER	SPECIES	Life cycle stage	SL (mm)	SPECIMEN VOUCHER	PHOTO VOUCHER
BLZ 7132	<i>Apogon mosavi</i>	A	35.0	USNM 401933	Yes
BLZ 7133	<i>Apogon mosavi</i>	A	24.0	USNM 401934	Yes
BLZ 7134	<i>Apogon mosavi</i>	J	18.5	USNM 401935	No
BLZ 7135	<i>Apogon mosavi</i>	J	17.0	USNM 401936	No
BLZ 7288	<i>Apogon mosavi</i>	L	16.0	USNM401937	Yes
BLZ 7711	<i>Apogon mosavi</i>	L	9.0	No	Yes
BLZ 7713	<i>Apogon mosavi</i>	J	15.5	USNM 401938	Yes
BLZ 7714	<i>Apogon mosavi</i>	J	17.0	USNM 401953	Yes
BLZ 7739	<i>Apogon mosavi</i>	J	17.0	USNM 401939	Yes
BLZ 7771	<i>Apogon mosavi</i>	L	15.0	USNM 401940	Yes
BLZ 7772	<i>Apogon mosavi</i>	L	15.0	USNM 401941	Yes
BLZ 8243	<i>Apogon mosavi</i>	A	28.0	USNM 401942	Yes
BLZ 8244	<i>Apogon mosavi</i>	A	31.0	USNM 401943	Yes
BLZ 8245	<i>Apogon mosavi</i>	A	30.0	USNM 401944	Yes
BLZ 8246	<i>Apogon mosavi</i>	A	28.0	USNM 401945	Yes
BLZ 8247	<i>Apogon mosavi</i>	A	23.0	USNM 401946	Yes
BLZ 8386	<i>Apogon mosavi</i>	A	25.0	USNM 401947	Yes
BLZ 8387	<i>Apogon mosavi</i>	J	15.0	USNM 401948	Yes
BLZ 8388	<i>Apogon mosavi</i>	L	14.0	USNM 401949	Yes
BLZ 8476	<i>Apogon mosavi</i>	L	17.0	USNM 401950	Yes